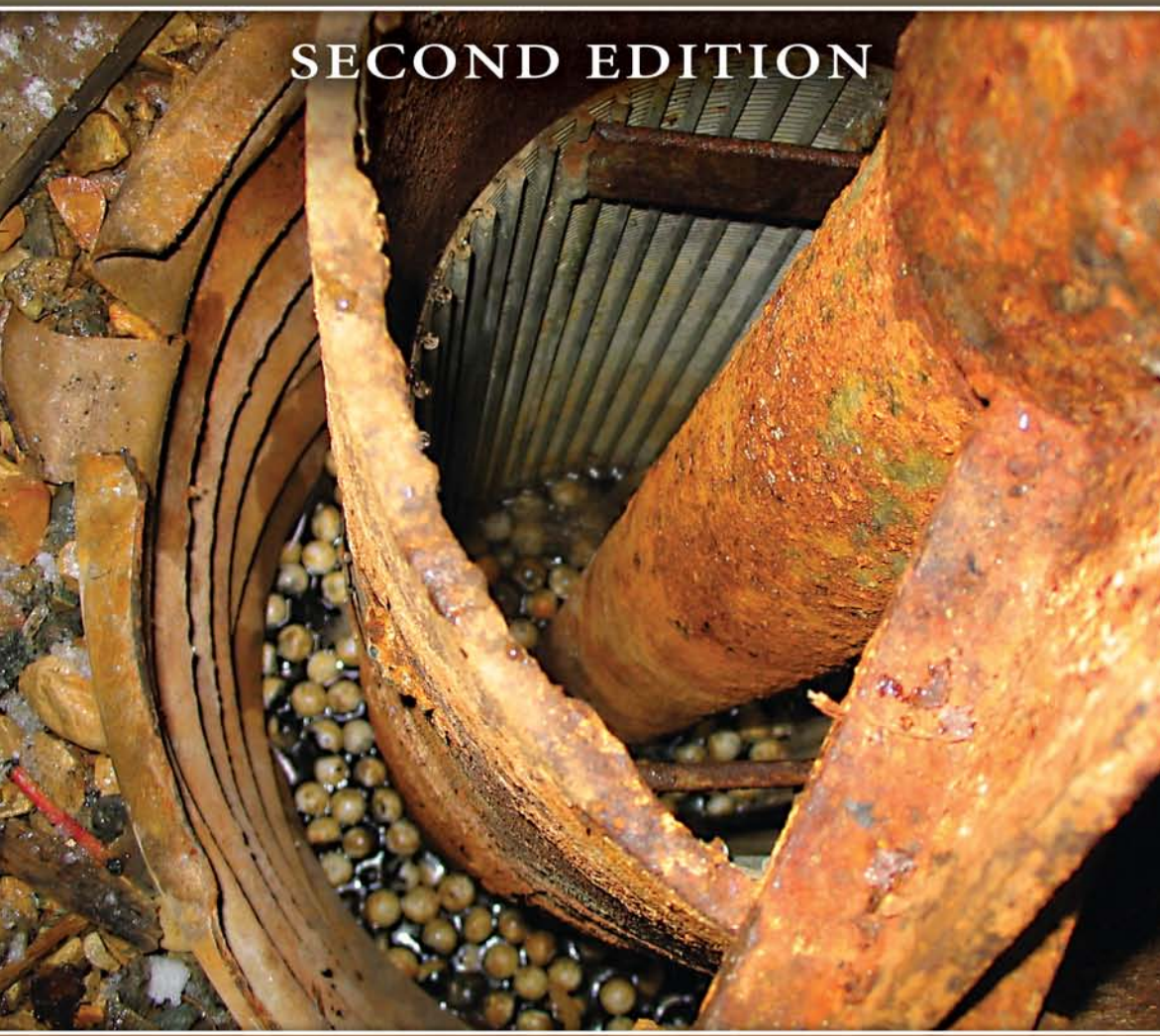
Practical Manual of GROUNDWATER MICROBIOLOGY

SECOND EDITION



D. Roy Cullimore

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The Sustainable Water Wells Series

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GROUNDWATER MICROBIOLOGY

SECOND EDITION

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Preface

It has been 14 years since the first edition of *Practical Manual of Groundwater Microbiology* was first produced by the Lewis Publishers and went on to become a bestseller. Although I have authored, edited, or otherwise contributed to a number of books, I did not want to tackle a second edition of the practical manual until there had been some progress. This book has had additional challenges that were numerous but hopefully will lead to a better read, or at least referred to, by a wide spectrum of people from water well operators to consultants, scientists, engineers, regulators, and even the interested microbiologists. One common feature most of the readers will have is that they will not have a degree in microbiology (let alone in microbial ecology!) and so the writing has to be aimed at a very diverse group. Because of the diversity expected in the readership, this book is not being written as a scientific document containing a myriad of references. It is, however, written as a "scientific opinion" that I have developed over more than 40 years in dealing with microbes in waters, soils, porous media, and all manners of natural microbial events. There have been many radical changes as this book progressed through the red pen stage, and now it is in three sections. Chapters 1–8 give an overview of microbiology and its importance in groundwaters. Chapter 9 is really a stand-alone guide illustrating separately 79 important concepts that are integral to the understanding of microbes in the groundwater. For those who want to pursue information in more detail, Appendices A–Q are specific to more specialized topics. Appendix R follows as a glossary for the vast array of words that may not be familiar to the reader. Appendix S gives the format for a simple questionnaire that is already used with clients and allows a better assessment of the risks presented in the management and operation of specific water wells. Finally, Appendix T includes potential further reading and references for those who wish to delve more deeply into more specific sources of information.

Unfortunately, the microbiology of groundwaters has never been at the center stage of economics and politics. As a result, it has remained an often poorly understood backdrop to the events occurring in groundwater and water wells. What has to be remembered is that microorganisms are virtually everywhere from the clouds above down to the soils, oceans, rivers and lakes, and even into the deeper geological formations present in the upper part of the crust of the planet. Groundwater can, by some, be considered as the "living" blood of the planet, since it forms a natural conveyance for microorganisms present and active within that environment. This book concentrates on the manner in which these microorganisms affect the groundwater. In particular, how their activities impact, often very significantly, on water wells.

In summary, in the last 40 years, microbiologically influenced events have had some very significant impacts on groundwaters causing clogging, plugging, formation of concretions, generation of gases, development of corrosive processes, taste and odor problems, diseases, and even the generation of electromagnetic potentials. Combinations of these events have also resulted in the failures of well fields, dams, catastrophic destruction in equipment as well as the generation of infestations. These

infestations have not only threatened humans and livestock but also even relatively pristine well fields. Just as a surgeon will make every effort to prevent cross-infection from one patient to another, likewise the water well operator needs to ensure that cross-infections do not occur between wells. Cleanliness is next to godliness, and this has to be practiced by operators to ensure that the dirt from an infested well does not bounce off into a clean well!

Perhaps we are at a severe disadvantage when "looking" at the microbiological aspects of groundwater and water wells because it is not possible to be able to "see" the biomass beast that is lurking down there out of sight in the well. Biomass is the name I will be using throughout the book to describe the complex communities of microorganism that are active and growing in the groundwater particularly in water wells or natural springs. Other terms include: biofilms that are made by groups of like-minded microbes that are being active in a small community structure usually as slime on a surface; redox front that refers to the "jungles" on the edge when oxygen is disappearing and respiration has now become much more challenging; and communities (consortia) that are the groupings of many different species of bacteria that cooperate for the common good. In reality, there are very rarely cases when a single microbial species will totally dominate a given environment. There is always some level of interdependence between these species to the point that it is much more significant to identify the nature of the communities rather than the biochemical and genetic specifics of a given strain.

In examining the microbiology of communities within the groundwater and the many surfaces to which attachment can occur, there is a need to identify the communities that are involved and measure how active (or aggressive) these communities are. After years of frustration with agar spreadplates and traditional bacteriological practices, we invented the biological activity reaction test (BART™), which is able to qualitatively define the communities and semiquantitatively determine the populations. This test has now become a standard practice if you want to get better information on the biomass beast.

Water wells lose production over time, which used to be linked to geological and chemical events but not biological! The first water well confirmed to be plugged with microbial activity was treated using steam injection in 1974. It had been flowing at only 5 gallons per minute, and after the steam treatment, which took the well up to greater than 85^{o}C, the well rate recovered to 12 gallons per minute, which was as good as the well has ever been (but nobody kept specific capacity, Q/s, records at that time). Two years later, we treated a Ranney well that included 12 wells that appeared to be biofouled with iron bacteria. These wells had lost production and were flowing at a combined rate of about 90 gallons per minute and the local users were getting ready for a legal wrangle over who should get that meager amount of water. Three of the horizontal wells were steam injected, and at that time we got an extra 12 gallons per minute and the water was bleeding bright red. We left with the cloud of failure hanging over our heads. Ten days later, the water coming from the well was crystal clear and flowing at 300 gallons per minute, and the legal wrangling quickly stopped. It appeared that the bacteria plugging the wells did not like heat, and indeed heating the well had cooked the biomass beast and it was gone leaving the wells flowing again. Ten years later and now with many

failures, it became clear that heat can coagulate the biomass into an impenetrable plug around the well, which no one can remove. It was brought back to the laboratory and a combination of heat and BART testers showed us the path. Since 1986, much of the experiences in the groundwater microbiology relate to the rehabilitation of water wells.

Major successes have been achieved rehabilitating plugging water wells through a blend of chemistry and heat (patented as BCHT™) and now over 6000 wells have been treated, and the latest city to adopt this treatment was the city of Fredericton in New Brunswick, Canada. As more wells have been rehabilitated, many clients find the word difficult because injured workers are also rehabilitated and there are hidden costs. For this book, I am moving away from the word "rehabilitation" to the word "regeneration" because the object of a radical well treatment should be to regenerate the well to its original characteristics.

Words are critical to the understanding of any discipline and for water wells that do degenerate; the other critical set of words is "preventative maintenance serving" (or PM for short). Because you do not get to see around the borehole outside of the casing in the porous media or fractures, it is not easy to appreciate these growths. In practice, water wells should be treated the same way as cars. Both need routine servicing as a part of the preventative maintenance strategy. All water wells should come with an owner's manual that specifies the steps that need to be taken to ensure that the well is truly sustainable and worth the investment.

There are some practices that I would like to see strictly regulated, which would not make me popular with the groundwater biomass beasts. It is an inevitability that sooner or later we are going to regulate, if not stop altogether, the use of phosphorus as a component in well treatment methods. This is because we are essentially feeding the "beast" so that it will then grow even more actively than before. Phosphorus is, to the microorganisms, like gold to humans only even more so because phosphorus provides one of the basic building blocks for high energy storage (as ATP). If we can control or totally prevent the use of phosphorus in downhole treatment of wells, then the biomass will become that much weaker, particularly after a treatment, and the wells may now last longer.

This preface provides but a summary of the many microbiologically influenced events that can occur in groundwater, which, when managed and not ignored, should allow more confident use of the groundwater. This applies beyond water wells to oil and gas wells and also to the practices involved in bioremediation where natural microorganisms with or without supplementation are able to accelerate the degradation of organic chemicals of concern.

This book would not have been written if the concepts and management practices discussed in the book had not been developed. There are many who helped in constructive manners in the development of the ideas and the subsequent manuscript that you are now reading. Appendix T includes the works of many of the people with whom ideas were developed, methods tried, and successes achieved or denied as nature intended. First, I would like to acknowledge the support of George Alford (ARCC Inc., Port Orange, Florida, U.S.), who was the coinventor of many of the patents, and has a deeper insight into the functioning of water wells. To all of the staff of Droycon Bioconcepts Inc. (DBI), I would like to express my deepest thanks

for their unbridled criticisms and creative suggestions that make DBI what it is today. There has been considerable enthusiasm over the years to make water wells more sustainable, and both the Prairie Farm Rehabilitation Administration of Canada Agriculture and Agri Food, and the cities of North Battleford and Fredericton, have, at different times, been the major demonstration sites for the sustainability initiatives. In the U.S., the U.S. Army Corp. of Engineers has been very supportive, particularly of the needs to manage biofouling risks in relief wells and on hazardous waste sites. In particular, the late Steve White of the USACE was very supportive of the needs of managing the microbiological influences that were impacting all types of wells. Additionally, I would like to thank Vincent Ostryzniuk and Jason Cullimore for their preparation of the figures shown in Chapter 9. Also I would like to acknowledge the interest of Neill and Gunter Ltd, Canada, for developing the questionnaire that is included in Appendix S, and Luminultra™ Technologies Ltd, Fredericton, Canada, for the use of their methods in Fig. 26 (Chapter 9). In Canada, financial support for some aspects of the research and development presented in this book was obtained from the National Research Council of Canada through a number of their industrial research assistance programs along with the research and experimental development tax credits. This support from the Federal Government of Canada allowed the successful development of research initiatives that could not have proceeded any other way. This support is also acknowledged.

This book addresses one of the biggest biomasses that reach downward to the magma and upward into the soils above. It is thought that this biomass is dominated by bacteria that have tremendous abilities to adapt to very hostile environments. Again, I apologise to the biomass for wanting to take away their supplies of phosphorus that are presently being applied to treat some of the problems that these organisms are, at least partly, responsible.

D. Roy Cullimore

Author

D. Roy Cullimore was trained in England at the University of Nottingham, School of Agriculture. He moved on to teach for 6 years at the University of Surrey before moving to the University of Regina where he became the director of the Regina Water Research Institute and a registered microbiologist in the Canadian College of Microbiology. Roy retired in 2001, but is an emeritus professor in the Faculty of Engineering at the University of Regina. He is very actively involved in Droycon Bioconcepts Inc. (DBI) as a consultant and manager. In 1996, he dove to the shipwreck of the *RMS Titanic* and was the first scientist to place tests on the ship, which would help identify the types of bacteria involved in the biodeterioration of the ship steels. Further expeditions in 1998, 2001, 2003, 2004, and 2005 advanced the understanding of the rate at which the ship is deteriorating. Deep oceans, as well as deep wells, are now a major activity at DBI, and ongoing research is underway, particularly with the shipwrecks and gas hydrates in the Gulf of Mexico.

Today, Roy spends time between operating DBI as the main manufacturer of BART products, and undertaking research, experimental development, and consulting. In all of these activities, the prime focus has been applied microbial ecology. This means efforts are directed at microbiological problems in high-level nuclear waste disposal facilities, gas hydrates (mostly in the Gulf of Mexico), oil and gas well regeneration, diagnostic technologies for biofouling water wells, corrosion of steels and concretes, and the development of novel microbiologically influenced processes.

1 Water Wells Have Natural Filters: The Challenge

1.1 INTRODUCTION

"It is just a hole in the ground!" is the way some people talk about a water well. They see the groundwater being pumped up as "good" water or "free" water. Something that nature has provided at no charge! Reality is that there is always an impact. Taking the water out of the aquifer depletes it of that water; putting a bore hole in the ground to remove the groundwater impacts on the soil and rocks; and packing and grouting the well all create impacts. But the water well is now in a postimpact state and pumping water in a predicted manner makes everyone happy.

What has actually happened is that a route has been created through which groundwater in the aquifer is now being pumped to the surface. This groundwater is subjected to a number of events as it enters into the sphere of influence of the pumping well. First, the water slowly begins to flow faster with increasing velocity as it approaches the well. Second, the water now begins to tumble and swirl as the flow rates continue to speed up closer and closer to the slots or perforations in the screen or casing of the well. Once inside the well, the water is commonly pulled towards the pump or less commonly bypasses the pump and floats for sometime in the water column of the well. During this whole process, there is another change occurring where oxygen is now appearing in greater quantities in the water. This oxygen causes the microbes that require oxygen to be much more active along what is known as the oxidation–reduction potential (ORP) gradient. As the water approaches the well, it moves from a reductive (no "breathing") to oxidative ("breathing") state from the microorganisms point of view. This transition is called the "redox front" and it is here that most biologic activity occurs within a well. It is also here at the redox front that the biomass forms. It is this biomass growing in and around the well that creates the natural filters that, for a period of time, improve the quality of the water.

People in general do not realize that the pumped water from a newly developed well is filtered. The common thought would be that the water is good because it has come straight out of the ground. There is not a general appreciation that aquifers are alive with the activities of microbes but they are; and the controlling factors are food, oxygen, and living space (does that sound familiar?). The search for food, oxygen, and living space causes the natural filters to form.

To understand the nature of these filters, perhaps the easiest thing to do is to follow the water as it moves from the aquifer into the sphere of influence of the

pumping well and onwards (and upwards!) until it is pumped out of the well. Groundwater is very different from surface waters, which only have limited contact with surfaces. Groundwater moves through voids surrounded by surfaces to which microbes attach and grow. It may be difficult to believe that microbes do not just float about in the water. Microbes generally like electrically charged surfaces that are anodic and dislike surfaces that are cathodically charged. That is one reason why steel pipes and ships are often cathodically protected to stop the "little critters" from attaching. While there is always some life (biomass) in an aquifer generally attached to the porous media and fractures, the amount of activity is very low until the environment changes. One major effect causing this change is the redox front that forms wherever oxygen is entering into the groundwater.

Reality is that the redox front becomes a battle ground where the microbes fight for the oxygen (oxidative side) coming in, and around, the well; and for the food which is being carried by the more reductive groundwater and can be broken down once oxygen is present. The food is coming from the reductive side and is dominated by the types of organics that cannot easily be degraded in the absence of oxygen. Such organics include methane (a major component in natural gas), hydrocarbons, and reductive septic wastes. Once these organics enter the oxidation zone, they can be degraded quickly by the microbes that use oxygen, creating carbon dioxide and growth of the biomass. Much of the growth therefore occurs at the redox front at the furthest limits of the penetration of oxygen into fouling media and closest to the food carried by the groundwater moving into the active well.

Growth now occurs within the void spaces at the redox front. This growth occupies some of the void volume and that can change the water flow rate through the redox front. Not only will this growth change the flow by forming a plug, but it will also be removing food and other "useful" materials from the groundwater. The net effect of this would be that the downstream groundwater that had passed on into the well would have been filtered through the one or more natural filters surrounding the well. There could be more than one filter arranged as a series of growth cylinders around the well, each of which occupies a particular place within the redox front.

Groundwater passing through a young natural filter is therefore going to be influenced by a number of activities that are happening within the water as it moves from a reductive condition to an oxidative state. It is not only the organic chemicals that are utilized by the growths in the voids but also many other chemicals. Those chemicals that are filtered out by the microbial growths are no longer in the groundwater entering the oxidative side of the redox front. There are a number of specific effects relating to the growth of a biomass at the redox front. These can be summarized as being separated into two major effects:

1. Degradation.
2. Accumulation.

Either of these functions removes chemicals from the groundwater through a natural action.

Degradation involves the breaking down of the chemical into smaller molecules with the release of energy. In the presence of oxygen, these smaller molecules could

include carbon dioxide and water (if the chemical is organic and has been degraded completely). Some of the organic molecules would not break down completely but be used by the microorganisms to construct cellular material or polymers. Polymers are long stringy molecules outside of the cells that perform a number of functions. Two prime functions are protection and storage. Protection is provided by the polymers forming a buffer around the cells and holding water in place (that is where the word slime comes from). Storage comes from the fact that the microbial cells are so small that they store much of the chemicals they may think of as being useful outside of the cells tangled in with the polymers. This storage is a biologic accumulation of chemicals that exceed the ability of the microbial cells to take them in. Bioaccumulation takes the form of storing reserves that the cells cannot use right now, taking up chemicals that may be toxic or useable sometime in the future, and allowing future microbial generations to be able to grow under stressed conditions.

There is a series of natural filters around the well that remove various chemicals, and can improve the quality of the water being delivered from that well. This will occur until the growth in the voids inside and around the well, as well as inside the bore hole, begin to mature. With maturation, the whole biochemistry of the water wells filters change from being the role of filtering out chemicals to the role of sequential releasing these same chemicals that have accumulated. At this time, the water quality will begin to degenerate and at the same time, the production will slow down as the voids "foul fail" (this term is used to describe the well failing because of biofouling). The alarm bells should have gone off once the water quality started to go down and the specific capacity of the well begins to fall. What chemicals may be considered markers for the success and then failure of the water well? Each chemical has its own distinctive pathway through the natural filters created around the water well. These will be addressed in turn taking into account that there is biofouling in the well that is causing the creation, and eventual failure, of the natural filters.

1.2 FEEDING THE NATURAL FILTERS

Organics form a natural and primary feedstock for the microbial biomass since it provides a source of carbon (50% of a living cell is carbon) that can be used to form new cells or as a source of energy. There are a myriad of organic chemicals in the world and some are more easily digested than others, particularly under oxidative conditions. Popular organics that are on the top of the gourmet list for microbes are small simple carbon molecules such as glucose (yum yum!), methane, fatty acids, and proteins. These are generally accumulated efficiently and used rapidly. Other organics tend to bioaccumulate and are only degraded if there are no more easily digestible organics available. These tend to be the carbon polymers (long stringy molecules) with cellulose (found in most plants) and hydrocarbons (found in crude oil) being the most common of the longer surviving (recalcitrant) organics. These recalcitrant organics may not even be bioaccumulated but flow on through the system untouched.

Phosphorus to the microbes is as important as gold is to humans. It provides a means to store energy (microbes do not have lead acid batteries but they do have

adenosinetriphosphate (ATP) in which the phosphorus can be stored as a high-energy reserve). Due to this feature, microbes will go to great lengths to store more phosphorus than they really need. To do this, most microbial cells will take up the phosphorus and "weave" it into complex stringy polyphosphates where the phosphorus is stored for future needs. Even if there were no phosphorus at all in the environment, these polyphosphates could then be used to support growth for five to nine generations (that would mean a single microbial cell would be capable of producing 32–512 cells just using these polyphosphate reserves).

Phosphorus is therefore a form of "fertilizer" when applied to water since it can stimulate so much microbial activity. This was one of the principle reasons why phosphorus-based detergents were banned some three decades ago to control extreme levels of microbial (algal) growth in surface waters. Even today there are still lakes and rivers recovering from this excessive application of phosphorus! For producing water wells, the level of phosphorus in the water should be very low if the natural filter is functioning and has not reached a state of collapse that accompanies the maturation of the plug/slime/encrustation/tubercle. Even today, some well treatments recommend that you do include significant levels of phosphorus. It would be reasonable to expect that this phosphorus would also be bioaccumulated by the survivors of any such treatment thus setting the "seed" for even heavier growths in the biomass after treatment. It is logical that, at some time, the rules governing the ban on the use of phosphorus-laced products, which is currently applied to discharges into surface water, will extend to the treatment of groundwater. Eutrophication in a lake may be easy to see because of the intense algal growth and the occasional suffocated fish floating on the surface but for the water well how is it possible to directly see such intense microbial growths? Today, there are microbiologic methods to determine the level of these growths and their effects.

Nitrogen is an essential part of all proteins, DNA and RNA but, unlike phosphorus, it is very available forming 80% of the atmosphere as dinitrogen gas. It also saturates the waters including deep-ocean and deep-aquifer environments. Nitrogen is required in greater amounts by the living cell than even phosphorous and, commonly, the ratio of nitrogen to phosphorus is between 2 and 4 to 1. Nature has played different tricks with nitrogen making it more available through the direct fixation of the dinitrogen gas into ammonium (nitrogen fixation) that can then be used to "grow" proteins, DNA, and RNA. If there are very oxidative conditions (i.e., free oxygen present) then the ammonium can be oxidized to nitrate (nitrification) and, under reductive conditions, that nitrate can be reduced back to dinitrogen gas (denitrification). These activities mean, in practice, that there is a constant exchange of dinitrogen gas from the atmosphere down into the surface (and subsurface) biosphere via ammonium. There are many microbes capable of fixing dinitrogen as ammonium and also denitrifying the nitrate back into dinitrogen gas, but only a few are capable of oxidizing the ammonium to nitrate (nitrifying bacteria) which only occurs in the presence of oxygen. It can therefore be argued that there is always an abundance of nitrogen in the environment and biomass generation is not commonly controlled primarily by the amount of nitrogen. Nitrogen fixation is,

however, an energy-expensive process and microbes will avoid fixing nitrogen unless it is essential and there is enough available energy.

Sulfur is one of the major nutrients that has received less attention even though it is required by living cells at about half the requirement for phosphorus. Sulfur is very common in the groundwater as sulfate which is sulfur in its most oxidized form. Under reductive conditions, the sulfate can become reduced to sulfur or more commonly sulfide. There are a range of microbes that will reduce sulfate to sulfide (sulfate-reducing bacteria or SRB) as hydrogen sulfide ("rotten egg" odor), and other microbes that will reduce the sulfur-containing proteins with the release of hydrogen sulfide (anaerobic heterotrophic bacteria). Hydrogen sulfide is gaseous and very reactive with metals (particularly iron) leading to the generation of black metal sulfides. Commonly the presence of blackened surfaces and smelly slimes can indicate that conditions are reductive and that hydrogen sulfide has been microbially generated. On some occasions within the redox front, sulfur is taken to the elemental (yellow) form by some microorganisms. When this happens there can actually be granules of sulfur sitting either within the cells or in the tight polymers surrounding the cells (*Beggiatoa* is one bacteria genus capable of biologically refining sulfur in this manner). Commonly sulfur is not a limiting factor to the creation of a biomass (plugging) around the water well although problems can arise if hydrogen sulfide is generated within the filter's biomass. Here the symptoms could include the smell of "rotten eggs" and the generation of black slimes and surfaces (wherever there has been a reaction with the hydrogen sulfide still under reductive conditions). Under oxidative conditions, there remains a potential for these objectionable black sulfides to be oxidized back into sulfates by the sulfur-oxidizing bacteria (*Thiobacillus* contain a number of bacterial species capable of doing this). On some occasions, both the sulfides and the elemental sulfur can be oxidized to sulfuric acid that can have very significant environmental impact because of the acidic leachate. In water wells, it would be very unusual for the pH (acidity) to be affected to that extent.

Iron is an important element particularly in animals, where it is involved in the transfer of oxygen through hemoglobin in blood. In the biomass forming in, and around the water well, one of the important characteristics of iron is that it can be bioaccumulated. If there is a mild steel casing in the well, it is common for the iron to be gradually extracted from the steel (weakening the casing) and, deposited within the biomass. Iron in the groundwater in the dissolved, reduced form as ferrous iron, will also be bioaccumulated within the biomass of the natural filter usually on the oxidative side of the redox front. Here the ferrous (soluble) form of iron is oxidized to the ferric (insoluble) form, where it continues to accumulate. It is the iron-related bacteria (IRB) that are thought to be primarily responsible for these build ups of ferric iron within the biomass. This build up of iron creates a biomass that gradually converts from a dominance of slime to a dominance of iron-rich encrustations as the dried weight of iron moves upwards from 5 to 30%. During this phase, iron moving with the groundwater is most likely to be oxidized and bioaccumulated within the biomass but once encrustations form then some destabilization can occur. This would mean that the efficiency of the natural filters now begins to collapse and iron now bleeds through the filter into the well. For the operator, the sudden appearance of iron in the product water would mean that the filters are becoming saturated and

the system is now more likely to fail. Commonly there would be a series of spikes of increased iron content (as the biomass begins to collapse) before the iron content stabilizes at a higher concentration. While iron is the most common metal to be bioaccumulated, other metals (such as manganese, arsenic, chromium, zinc, and copper) can also be released from the bioaccumulates but generally at different times depending upon the nature of the redox front creating the focussed biomass in, and around the well.

1.3 OTHER FACTORS ASSOCIATED WITH NATURAL FILTERS

There is more to the water well than just the biomass that clusters around the redox front and forms a natural filter. It can be argued that the biomass activities down the bore hole and around the well provide a benefit (through the filtering out of chemicals from the groundwater) and a cost once the biomass becomes unstable causing the periodic and later continuous releases of the bioaccumulated chemicals into the product water from the well. These events are mostly microbiologic in origin but other well problems can be categorized into four broad problem areas:

1. *Structural or mechanical failure* can occur due to the natural physical stresses exceeding the designed capacity of the equipment. These failures exist either because of a failure to design and/or install the wells properly, or because the wells were impacted by some shifting in the soils and/or the geologic strata in the region. This group of problems is most likely to be well-specific events that do not involve either clogging or plugging activities. Improper development, poor design of filter packs or screens, or over pumping can cause physical clogging of extraction wells. Problems in the treatment processes applied to the water prior to injection can cause physical clogging in the injection wells.
2. *Clogging* due to geochemical impacts is dominated by physical and/or chemical factors. This type of geochemical clogging is driven by a mixture of materials created by chemical interactions that occur in the void spaces around the well now becoming filled by materials such as clays, silts, and sands (fines) that clog the voids as they move towards the well with the groundwater flow. It was historically thought that clogging was the major cause of well fouling and that it commonly involved an oxidative precipitation of chemicals, in particular ferric iron and carbonates, into insoluble accumulates. Changes in physical conditions in the well caused by turbulence often affects pCO_2 and/or O_2 leading to mineral precipitation. Sand, silts, and clays were thought to accumulate and cause clogging. Presently it is recognized that many events previously characterized as geochemical clogging has been microbiologically driven plugging events.
3. *Plugging* that is due to the microbiologic infestation of the well and/or the surrounding environments are caused by excessive levels of biomass.

Biologic plugging can start with the initial growth of biofilms (slimes) at sites where the growth now restricts groundwater entry into the bore hole. This initial growth matures and hardens through the process of bioaccumulation particularly of iron and other metallic cations, and through the synthesis of crystalline structures usually based on carbonates. At the same time, as this maturation occurs, there is also an entrapment of clays, silts, and sands that can add bulk to the plugging biomass volume. This entrapment then increases the losses in groundwater flow through the infested region to the well. Here the natural filters have moved from being beneficial (through the filtering of the upstream groundwater) to being a problem (through the releases of bioacccumulated chemicals into the downstream groundwater).

4. *Corrosion* can be related to microbial activities that cause an impact on the well screen and pump integrity. Corrosion can involve two major microbial groups. Electrolytic corrosion is triggered by the generation of hydrogen sulfide from either the SRB or the anaerobic heterotrophs that are also generating hydrogen sulfide. This form of corrosion can lead to pitting and embrittlement of steels and some alloys. Anaerobic heterotrophs can also produce short-chain fatty acids that can drop the pH into the acid range (acid-producing bacteria, APB) and cause corrosion of some metal alloys and concrete. Commonly microbial-influenced corrosion is grouped with plugging since, on most occasions, the corrosive processes follow the generation of a biomass that would commonly be contributing to a biologic plugging process.

1.4 MAINTAINING NATURAL FILTERS AROUND WATER WELLS

There are advantages and disadvantages to the formation of these natural filters around the water well. The advantages are that the biomass clustered mostly around the redox front, is removing chemicals from the groundwater flow coming from upstream and producing better product water. This advantage is slowly lost as the biomass grows and begins to plug the well. Maturation in the plugging biomass causes sloughing to occur. This causes releases of the surplus bioaccumulated chemicals back into the downstream water. With this happening, the advantage of the filter turns into a disadvantage for the well operator. The art now becomes finding a treatment methodology that would recover the product water quality and flows rates and, at the same time, return the natural filters to a useful condition. Treatment therefore has to involve the destruction and removal of the biomass that is plugging up, and/or corroding the water well, along with the surplus bioaccumulates. Ideally, the natural filters would be returned to a functional and advantageous state at the end of the treatment. That does not mean you either "make a new hole or poison the bastards!" (This conceptual expression has its roots in Australia). A new hole

comes with a price tag and the use of poisons may not be tolerated particularly for use in groundwaters that may be extracted as potable water.

Natural filters will recover from extreme treatments even if all of the local microbes are killed and removed from the sites in, and around, the well. This is because there are many more microorganisms out in the formation beyond the sphere of influence of the water well. These microorganisms will come into the wells environment and colonize the surfaces "polished" by the effective treatment of the well and a new set of natural filters will be created. In selecting a treatment process, it has to be remembered that it will have an impact on the natural filters already functioning around the well and there would also be the release of bioaccumulates from the well during the treatment process. On some occasions, these releases will contain very high concentrations of metals and recalcitrant organics that may require careful disposal. Effective removal of the biomass and the bioaccumulates from the treated well automatically means that the natural filters have now been "cleaned." For the treated water well, it also means that there is now significantly enhanced capacity to filter the upstream groundwater and produce an acceptable downstream water quality.

1.5 FAILURE OF NATURAL FILTERS AND SYMPTOMS IN WATER WELLS

There can be more than one natural filter in a water well that is impacting in both positive and negative ways on the water quality and quantity that is being pumped out of the water well. Initially, consideration should be given to the fact that these filters are not installed at a fixed site but are capable of moving laterally both closer to, or further away from, the bore hole. Additionally, these filters can move vertically up or down the bore hole. This movement is primarily responsive to the manner in which water is flowing into the well in either a laminar or turbulent flow. Laminar flow will tend to even out the growth of the biomass associated along the length of the bore hole while turbulent flow may cause the biomass to become focussed over that part of the well where there is a maximum amount of turbulent flow. The biomass that forms the natural filters is also very responsive to changes in the ORP with different parts of the biomass tending to focus at different sites along the ORP. A redox front is created where the biomass becomes more focussed when the ORP moves from a positive (oxidative) value of $+50$ mV down to a negative -100 mV. Different microbes tend to cluster around different parts of the redox front based upon the local ORP. Moving from the oxidative to the reductive side of the redox front, the dominant bacterial group will shift from IRB to slime-forming bacteria and heterotrophic aerobic bacteria (HAB) often with SRB associated, and then in the more reductive regions would still be SRB and denitrifiers. On the reductive side of the front, particularly where the ORP is in the range of -150 to -250 mV, the dominant bacterial group is likely to be the methane-producing bacteria (MPB). Where methane is produced by the MPB, either near to the well or at a distance in the geologic formation, then this methane is likely to be degraded within the redox front commonly in association with the aerobic

HAB. The other redox front that may critically impact the well is the front that can establish at the static water level within the formation. Here the groundwater conditions are semi-saturated with groundwater with gases (possibly including oxygen) permeating down from the overbearing porous media and the soils above. This redox front, extended laterally above the groundwater moving towards the well, can also impact quality through the movement of molds (fungi) that have been growing in that lateral redox area. These molds can release very high number of spores (small resistant cells) that can now enter into the natural filters surrounding the well.

The life cycle for these natural filters around the bore hole involves a number a distinct steps. First, there is an attachment of microbial cells to surfaces as a maximum amount of volume is occupied within that space to achieve dominance. At this time, there can be significant losses in water flow (transmissivity) but this is generally short lived ending with the surfaces becoming less resistant to flow (second event). The third event follows with compression of the biomass so the transmissivity now returns close to the original (uninfested) state. Fourth event is a period where there is a slow, progressive growth with the biomass functioning in a very natural manner going through, expansion, destabilization, compression, and stabilization. This harmonic action follows a distinct cycle like the heart beat but may take between 10 and 40 days to complete a single cycle. In the fifth event of the cycle, there is the gradual pulse growth now beginning to fill the voids and fractures around the well and causing significant losses in water flow (transmissivity). This is really an extension of the fourth event but here the growth suddenly causes a dramatic drop in the specific capacity (Q/s) and losses in transmissivity through the formations around the well. These sudden dramatic losses can be between 2 and 15% of the Q/s and happen over 1–4 weeks. This is followed by a relatively stable period that lasts three to six times the time it took for the dramatic drop in Q/s to occur. Over time, the plot of the Q/s will show a series of steps as the productivity of the well declines. The seventh and final event is the effective closing off of all groundwater flow into the bore hole. Such an event would mark a terminal plugging of the well and regeneration would now be extremely challenging and expensive.

Failure of these natural filters is therefore a progressive sequence of events that can be monitored to determine when preventative servicing and a full regeneration of the well would need to be applied. Essentially, the shift in the production of water from the water well would begin by instability in the Q/s as the water well moves through the second event phase. Once the well has been developed and is stable, both the quality and quantity of water produced by the well reaches a period of stability. Once the fourth event (gradual harmonic losses in flow) begins, it causes an impact not only on the Q/s, but also on the water quality. In this event, deterioration is commonly observed through increased turbidity (cloudiness), higher total suspended solids (TSS) and sporadic increases in particularly iron (when present) causing the water to shift towards a yellow color followed by red or brown which would also have high particulate content. Preventative servicing should be started as the Q/s begins to drop, with the objective of keeping the Q/s as close to its original 100% as possible. Losses in water quality can precede the loss in Q/s and can serve as an early warning mechanism when it is desirable to keep the well sustainable.

2 Microbes in Water Wells: The Good, the Bad, and the Ugly

2.1 WHAT ARE MICROBES?

We are all of about the same size as far as the human species go and we live clustered around, on top of the more solid bits of the surface of the planet called Earth. We are so preoccupied with all that we can see, touch, smell, and hear around us, but, we do not really want to realize that we are living within a myriad of bacterial gardens of life that extend deep down below us, grow up into the skies above, and outwards into the oceans flowing around our homes. These bacteria were "gardening" here long before we first evolved and set foot upon the soil and they will still be here long after, for whatever reason, we have become extinct. These gardens are a set of universes within which we can stroll through, belong to and pass through, often without the care of a single glance, or thought at the gardens stretched out for us all to see. In this chapter, we are going to concentrate on the good, the bad, and the ugly microbes that are down and around water wells.

It is important to appreciate that the world is full of microbes that have been changing and adapting just as much as we think we do but they have been doing it for a lot longer and it shows. No longer do I pause to think of "where are there microbes?" but rather "where are there no microbes?" This is because microbes are everywhere and are much simpler and much more adaptable. There is one huge advantage that these microbes have and it is that they work together so that while one species will do one thing, another species will do something else while they all share a common space in a slime/biofilm/encrustation where they can flourish together. We have been so preoccupied with plants and animals, each with their own space, that it can be difficult to grasp that microbes share and share alike. These communities are called consortia (or consorms) and may contain two, three, up to eight or more species all occupying that same common consortial slime.

Slime, now there is a word that immediately gets people thinking "horrible!" is the wrapping that protects many microbes from the world outside. We wear clothes and maybe an overcoat but they just wear slime. Unlike our clothes that are synthetic or natural fibers, their (the microbes) slime suits are all made of natural fibers (called polymers) that can bind very large volumes of water into the webbed weave of the

polymers. When we wear clothes, they are not very thick but just enough to stay warm and look suitable (for whatever the ego dictates). In volume, our clothes are less than 10% of our body's volume. This is not so for the microbes. Their slime suits can be 10–10,000 times larger than the microbe's cell size. Think of a microbe going in for a fitting and asking for a size 10,000 (that is four orders of magnitude bigger than the cell!). In perspective that would be like me wearing an overcoat that could be as much as a mile or 2-km wide! Remember that the slime suit for microbes is almost all water. We would look pretty stupid flopping around in a slime suit that was even just 10 times as big as we are, let alone more! Well, that slime suit serves the microbes in many ways apart from modesty! Already in your mind, there is this poor little cell flopped inside a suit of slime not able to move. Well maybe the cell does not need to move because everything it wants comes through the slime that also keeps back the nasty stuff that the microbes would not want to have touched their cells. So slime is a sieve. It is a case of "in with the good and out with the bad." The good would be the oxygen (if a microbe likes to breath and not all of them do), water (not all microbes are bathed in water all of the time), and food (yum and yum). Microbes like to chew on molecules more than we do because they are very small and have to literally "suck" all of the food through their cell walls (they have no mouth, no nose, and no eyes). Could you imagine a dinner party if we had to do that same thing? There we would all be wallowing in a bath full of soup or jumping into a pile of hamburgers. Of course to be delicate we would suck drinks up through a finger neatly placed into the glass! However, we do not need to do that because we shove everything into our mouths and keep the digestion out of sight and out of mind.

When the not-so-good things such as toxic metals gets bound up into the slime, if the microbes do not want it, then it may stay stuck there away from the cell for as long as the cells (collectively that is!) are alive. Sometimes the microbes may make use of nasty stuff. For example, they make use of iron as a food and they can also degrade it to make it usable for other species. Wandering around the world of plants and animals, you find that iron is an important part of many living systems down to even the very small micro-plants (algae) that have a need for much iron in order to grow. In the great oceans still on this planet, these algae are restricted in their growth by not enough iron. If these algae were to become more active through having more iron available then they would fix more carbon dioxide, control global warming and maybe even avoid the start of the next ice age! This text will probably only be read by a single species *Homo sapiens* var. *sapiens* and, for that species, iron forms a major function in moving oxygen around the body in the red blood cells. It is strange but by using x-rays, we have found that even rusticles (biologically formed concretions or living concrete) from the deep ocean appear to have what resembles a blood system through an interconnected system of iron-rich channels. Rusticles use most of the iron in the manner that we humans use calcium—to provide structural support. For humans, the calcium provides support on the inside of the living tissues as bones whereas for the rusticles, the support generated by the iron is on the outside of the living cells to literally create a home of rust. Rusticles can even grow in water wells, particularly those with steel casing or high-iron content in

the upstream groundwater. In some wells, video camera logging of the water well will make you think you are at the site of the *RMS Titanic*, which is a hanging garden of rusticles deep in the North Atlantic.

The bottom line is that microbes are everywhere including inside our bodies, in the deep oceans, granites, muds, clays, and clouds as well as everything in between. Even if you are a chemist, an engineer, a physicist, or an agronomist, microbes affect the jobs you are doing. Perhaps mathematicians are exempt from this, but unless they are using very clean stainless steel, plastic slide rules, or do all of their thinking in their head, then microbes can be a problem. If the mathematician uses a computer then that computer can be "attacked" by hoards of microbes looking for a very creative place to live. All of that electrical activity in a humid computer can form an ideal site for some forms of microbes.

2.2 WHERE ARE MICROBES?

Everywhere does mean everywhere unless it is very hot, so hot that there is no liquid water, for if there is liquid water then some microbes will find a way to grow regardless of the pressure! Because we live normally on the Earth's surface and wander around in the manner typical of most bipedal species then we tend to become hypnotized by the other life forms that we can see, bump into, avoid, and classify. When we think of the diversity of all of these plants (generally green and do not move fast anywhere) and animals (generally able to move about a bit and come in a variety of colors), we do not think too much about the diversity of the species of microbes.

Are you ready to jump into the water well to begin to explore? Looking down into a water well and ready to jump into the water you may notice that it does not look like the water in a swimming pool (all clear and clean). The surface of the water is rolled and folded into gentle curves looking like a liquid rubber mat floating on the water. What is really floating on the water is the first biofilms, a slime film, which formed the first "defensive" walls of the myriad of slime cities scattered far down below. This floating slime mat may be thin and did not offer any resistance as we dove straight through it into the water body below but what we are seeing now is not water (after all you cannot really see pure liquid water!) but slime that is everywhere. Chemists call these slime colloids and they are still at a loss to explain these in purely chemical terms. Microbiologists look at these colloids as small floating slime villages and drifting slime clouds that have peeled away from microbial biomass. This could be considered to be a kind of microbial "dandruff." These particles look rather like the clouds in the skies for they are fluffy. These microbial fluffy clouds are measured in microns unlike the clouds which are measured (when they are) in meters and kilometers (or miles). Unlike the clouds in the sky, these biocolloidal particles are much more stable and are occupied by fewer microbes. They are like the crew of a slime submarine, floating in the water on a journey to the future from the past and nothing more, and yet, what can be more important?

Biocolloids are almost transparent and often fluffy, made up mostly of water bound to the sticky filaments spun out around the microbial cells much like a spider spins a web to trap its food. For the microbes, the webs they spin traps water as well as food. The water that is trapped gives the particles the same buoyancy as water and so they can float. Water is full of this colloidal "dandruff" that are the small fractions of microbially spun webs that have broken away and yet still carry with them water bonded to the fibers (polymer is the word scientists commonly use to describe them) and with that water comes fragments of food. Like a fisherman hauling in a net to trap fish, these microbes use those web-like masses of polymers to trap the dandruff (fluffy food). Like the fisherman, they cannot trap just the foods they can use but they also net a lot of materials they cannot use. Some of these particles of unwanted flotsam and jetsam can be dangerous, or just simply useless. This unneeded flotsam/material is kept away from the cells by being locked up in the polymers but are kept there, in case one day a need should arise for them. Thus, the slime clouds become loaded down and heavier and yet do not sink but stay floating in the water controlling their density just as a submarine does. In the laboratory whole layers if these biocolloids can actually be seen to be floating as a cloud at one depth and then at another. This floating can be seen in some BART™ testers (see Appendix B for more details) when the water sample being tested shows these particles moving around or even floating plate-like within the fluids.

2.3 STERILITY, HEALTH, AND MICROBES

It appears to be the belief of the majority of the general public, many engineers/scientists, and some of the regulatory community that literally all bacteria and viruses are bad for human health. We (primarily North Americans) are using antibacterial/deodorant soaps and demand to drink "pure" water. The term "Pure water" is popularly considered to contain no microbial populations at all. This growing public demand to consume only "pure" (approaching a "virtually sterile" state) water is driving regulations that have had, and will increasingly continue to have, a costly impact on the production of potable water that is delivered by most public water supply systems in North America.

More than 20 years ago, most engineers and scientists considered groundwater to be "virtually sterile" and therefore in that sense "pure." It was generally believed, without a great deal of supporting science, that those microorganisms were "filtered out" of groundwater through the natural processes referred to as "advective intergranular flow." Moreover, it was also believed that the natural microbial flora was considered to have a short half-life, and thus could not survive during the normal slow movement of groundwater towards a well. When microbes were found in groundwater, these events were considered to be primarily a hygienic issue resulting from poor well construction and maintenance practices. Some of the problems extending from this dogma are the fact that microbial analysis of water supply has historically been, in most cases, limited to coliform bacteria as the prime indicators of hygiene risk. As a consequence of this zero-tolerance testing, large

public water supplies in North America have been disinfected to eliminate microbial (coliform) presence or growth.

Today, renewed interest and improved scientific methods have revealed that a wide range of aerobic and anaerobic microorganisms inhabit groundwater systems as their natural environment. These microbes include fungi, protozoa, bacteria, and viruses. Some of these microbes have the potential to be pathogenic (disease causing in humankind, if found in sufficient numbers), other microbes are beneficial (for example, assisting in the human digestive system), but the impacts and/or symbiotic relationships of a large portion of these microbes remains unknown. In reality, we have been unknowingly consuming these microbes in various quantities and proportions in water since the dawn of time and our immune systems are well aware of these microbes.

The issue that we need to address is, at what point, if any, do we begin to "overtreat" our potable water by removing too many of these microbes? We know today that trace amounts of minerals and metals found native in most groundwater are required for good health. If we remove these trace minerals and metals from the water, then it becomes necessary to supplement. By the same token, if we remove all or most of the microbes from our drinking water supplies, will we reduce our inherent resistance to other microbes (say the ones that we eat or breath in), and end up taking microbial supplements as well as mineral and metal supplements? Are we unknowingly generating conditions that could cause an engineered immune-deficiency syndrome (EIDS) by excluding these natural microbes from our bodies? What microbes have we been historically ingesting through our water consumption that is necessary to maintain good health? There has been little scientific attention given to this potential risk.

Most North Americans cannot go to many parts of the world and drink tap water without suffering from severe gastrointestinal illness. The cause of this nasty and sometimes serious medical condition is, for the most part, due to the lack of resistance we have to the natural and contaminating microbes present in the local water supplies. If we continue along the pathway of removing all microbes from our water supply by means of chlorination, ultrafiltration, or other treatment, will urbanites (those people drinking water that has been "overtreated" by removing all or most of the native microbes) then begin to develop EIDS, and not be able to drink untreated water? Is it possible that a trip to rural America in the future will require buying bottled "purified" water or importing your own drinking water just in order to stay healthy?

We know that microbes are found throughout the human body, and are very necessary for human survival. We also know that groundwater almost inevitably contains a wide range of microorganisms, which we have been consuming since the dawn of time. Until recently, research in the area of groundwater microbiology has been driven by the search for specific pathogens known to cause a recognized particular disease of direct concern to humans. Environmental remediation of contaminated groundwater has increased our knowledge of aquatic microbiology. Still today, there has been little research on the normal microbial flora found in groundwater, let alone those that could potentially be "healthy" for humans. Before we continue on the pathway of "overtreatment" of our potable water by removing all

or most of the microbial population, there is a need for the necessary scientific studies to be completed to determine the realistic nature of these potential impacts.

2.4 BACTERIAL GROUPS OF SIGNIFICANCE IN WATER WELLS

Water wells sit at sites where they are automatically likely to be infested with various types of microbial activity from the natural environment, as well as with microbes being introduced from the activities associated with the development of the well. Water wells can also be vulnerable to surface waters that move down into the groundwater carrying microorganisms with it. This effect is commonly referred to as "groundwater under the direct influence of surface water" or "Gudi" often pronounced as "gaudi" (sometimes also as GWI, groundwater influenced by surface waters). Here microorganisms from the surface can infest the water wells and this can include pathogens that may then create a health risk. In the operation of water wells, it is a priority to ensure that the water wells are creating water that is safe to drink or use. The marker microbes for indicating whether a health risk exists for the water well are the coliform bacteria. Therefore, these have become a preoccupation for water well operators to ensure that the wells are not presenting such risks. Commonly, the presence or absence of coliform bacteria does not have any significant bearing on the other risks to the water well that are more associated with water quality and production issues. The major bacterial groups that create other types of risks are iron-related bacteria (IRB, common where there are high iron concerns in the groundwater); sulfate-reducing bacteria (SRB, common where there is a corrosion risk or black slimes are occurring); denitrifying bacteria (where there is a risk of high nitrates or nitrogen-rich waste waters entering the well through a gudi or the groundwater); heterotrophic bacteria (HAB, where the risks come from high-organic loading that could be associated with a plume of organic materials such as gasoline, or methane being carried in the groundwater); and slime-forming bacteria (that generate intense slime-like growths and plugging within the well and hence is a direct threat to the production of water from the well). Each of these bacterial groups will be addressed in turn in the ensuing sections of this chapter and detection methods will be addressed in Appendix A.

2.5 COLIFORM BACTERIA

The name "coliform" is given to a whole group of bacteria, which can be found in water and indicate potential health problems. They are divided into two groups: total coliform (TC), which is all of the coliform bacteria; and fecal coliforms (FCs), which are that portion of the TC that have disease-producing (pathogenic) risks. Both of these bacterial groups are closely related inside a large family of bacteria known as the enterics. However, most of the enterics belong to the TC group, but very few belong to the FC group are considered much more serious from the hygiene viewpoint. In testing water for its hygiene safety, the presence of TC, and more particularly FC, spells trouble with a capital "T"!

The FCs are specialized types of bacteria and are dominated by *Escherichia coli* (Esh-her-ick-ee-ah co-lie), also known as *E. coli*. This bacterium thrives in the healthy human intestine and passes out in high numbers in the fecal material. These can be counted in water by using the FC tests and the counts are usually given as FC cells in 100 mL of water. Counting the FC is done by growing the bacteria under very specialized conditions, for example at higher temperatures (44.5°C), this discourages the TCs from growing at all or in a peculiar (atypical) way which the technician will ignore when counting the typical colonies. Human feces tend to have much more FC than fecal streptococci (FS), which some people look for as well as, or instead of, FC. These FS tend to be more common in animal feces and so comparing the numbers of FC to FS (FC:FS ratio), is considered handy for getting an idea as to whether the water has been polluted with human fecal wastes ($>2{:}1$ ratio) or animal wastes ($<1{:}1$).

Escherichia coli are commonly found in the human intestine, but it is not usually harmful. However, there are some strains which can cause clinical infections such as the strain 0157. What is important to remember is that the presence of FC is a widely accepted indicator of the potential pollution of water with fecal material. If that material is present, then there is a much greater risk of infectious microorganisms occurring in numbers large enough to cause an infection to break out if the water is consumed. These potentially pathogenic organisms include viruses, other bacteria, protozoa, and a variety of worms. There is some FC that so closely mimics *E. coli* even when cultured as 44.5°C that they also will grow at these temperatures. They are known as the thermal-tolerant coliforms and are much more closely related to *E. coli* than the FC group.

2.5.1 Total Coliforms

The TC group contains a wider variety of bacteria including *E. coli* and a broad spectrum of other enteric bacteria. These enteric bacteria are frequently able to grow in the intestine, but some can also grow to a variable extent in the environment. In consequence, the TC count does not necessarily relate specifically to fecal pollution or hygiene risk, but more to the bacterial loading of enterics within the water system. Since the count is dominated by the bacteria which can occur in the intestine, it is used as a broader spectrum test for fecal pollution in a water system. Frequently, these bacteria also like to grow in muds, septic wastes, and organic-rich wastes which can distort the numbers and findings. For example, when swimmers and bathers kick up the sediment and muds along a bathing beach, large numbers of TC can enter the water to give higher counts. The natural conclusion is that the daytime unsanitary habits of the bathers caused the elevation of the TC bacterial count when it could have been, at least in part, from these other sources.

2.5.2 Testing for Coliform Bacteria in Water

How can bacteria as small as one-thousandth of a millimeter, or 1 μm, be counted and at the same time identified? Good question! Bacteria are like big bags of reactive chemicals (enzymes) which have the habit of dividing into two bags when the bag

gets too full or things get too stressful! The counting procedures take advantage of these features and there are two principal methods used. These are known as the membrane filtration (MF) and the most probable number (MPN) methods. You got it, one method filters the water to trap the coliforms (MF), while the other makes a hopefully good guess (probability) at the number of coliforms present (MPN) by culturing the water sample.

2.5.3 MF Coliform Test Method

There are special filters which can trap bacteria in water by having pore sizes too small to allow the bacteria to pass through (0.45 or 0.22 μm). The bacteria are therefore trapped on the upper surface of the filter. For example, if there were only one bacterial cell in 100 mL of water filtered through the membrane filter, it would become entrapped into the top of the filter as the water passed through. Now, to see these entrapped bacteria, we could run a microscope over the whole field but most of the bacteria are colorless and would not be seen unless stained! What is better is to grow the single bacterial cells into large colonies containing billions of its offspring that could be seen as a visible single heap of growth called a colony. Bacteria, like human beings, prefer particular foods and are repelled by other foods. When the "menu" is properly adjusted, specific groups of bacteria can be made to grow and be stained (if stains are added) at the same time. Even when a colony grows into either a dome, pancake, spreading slime, a feathery rhizoid or some other pattern, those characteristics are typical for that species. In the coliform MF tests, very specific foods, selective agents and stains are made available either in a gel (agar) or in a liquid added to an absorbent pad. The nutrients and stains pass up into the filter pad placed upon the bacterial colony and, at the right temperature (35°C–37°C or 44.5°C), colonies for the TC or FC bacteria grow within 24 h and can be counted visually. If you know how much water you filtered and count the number of typical colonies, then you can calculate the numbers present in 100 mL. Other bacteria may also grow, but will not produce the characteristic (typical) colonies, but instead produce atypical growths, which are usually not recorded.

2.5.4 MPNs Coliform Test Method

All bacteria are like bags of enzymes and they selectively use different foods, particularly sugars. One of the favorite sugars for the coliform group is lactose. Strangely, that is the main sugar in mother's milk and, therefore, the first one that the bacteria in the baby's intestine come across when the baby is suckling the milk. Bile is lethal to most bacteria, but the coliform bacteria can tolerate it. Therefore, it was found that a soup rich in lactose and bile encouraged the coliforms to grow while discouraging competition (that cannot use lactose or tolerate bile salts). Not only that but also the coliform bacteria produce large quantities of gas when feeding on the lactose. It was Durham who first trapped this gas in an upside down test tube which was then named after him. When acids with or without gases are produced by bacteria, this reaction was referred to as fermentation. It is possible to use this fermentation to detect coliforms. For example, if you took 100 mL of water and

found that gas was produced from a 2-mL sample of water when the lactose bile broth was added, but none was produced from 1 mL, then that would suggest that the water contained 50 coliforms per 100 mL (i.e., enough for just one cell in every 2 mL of water). As you are probably thinking, there is an element of chance in that happening and so the statisticians took over and developed a fractionating series of tests to apply to the water sample that would allow a population to be predicted. This prediction is based upon which dilutions produced gas and is called the MPN of coliforms per 100 mL. If you are very lucky and the lab very honest, there will be a second number which will give the range of error. This number usually represents the range around the MPN within which two-thirds of the predictions of populations would in fact be accurate.

2.5.5 MUG Test

Fundamentally, the prime application of the 4-methyl umbelliferone glow (MUG) which is a signal important in the MUG test for the detection of coliform bacteria can be found in the IDEXX products (Westbrook, Maine). These use a patented Defined Substrate Technology (DST®) using two "nutrient indicators" which biochemically trigger the enzymes β-galactosidase when coliform bacteria are present and the enzyme β-glucuronidase when *E. coli* is present. Two nutrient indictors known as the *o*-nitrophenyl test (ONPG) since this and the MUG are administered to the Colilert test depending upon the target. For the coliform bacteria, they utilize the ONPG causing a color change from colorless to yellow when *o*-nitrophenol is produced. For *E. coli*, it is the MUG indicator that detects β-glucuronidase activity which generates 4-methy-lumbelliferone which is fluorescence pigment detectable using ultraviolet light. It is claimed that this approach is significantly different from the use of traditional media which provide a nutrient-rich environment that supports the growth of both target organisms and nontargets. The claims for the MUG test include that a few noncoliforms that do have these enzymes are selectively suppressed by Colilert's specifically formulated matrix. To suppress nontarget bacteria, traditional media often include high levels of salts, detergents, or other selective agents which may inadvertently suppress target organisms and give further false negatives. Today the IDEXX Colilert reagent is used around the world for the detection of coliforms and *E. coli* in water. Colilert, with its patented DST®, is used in most of the U.S. State labs. Colilert is, in fact, used more than all other methods combined (U.S.A., Canada, and Japan drinking water markets). It is U.S. EPA-approved and is included in *Standard Methods for Examination of Water and Wastewater*.

2.6 IRON-RELATED BACTERIA

Rusty slimes, plugging, yellow to orange waters, production losses from wells and blackened corrosive slimes have all been connected to the iron bacteria. These iron bacteria are usually found in waters having relatively high concentrations of iron and manganese in the water (greater than 0.5–1.5 parts per million for iron and 10 times less than that for manganese). When they grow, the slime can plug pipes, well

screens, pumps (particularly the impellers and screens), and can even grow back into the groundwater of an aquifer! Because they grow better when iron is present, they are often called IRB. There are three major groups of iron bacteria

1. *Gallionella* is the most distinctive group of the IRB in many ways. For the scientist looking into the water, this organism can be easily recognized by its long ribbon-like tail which is produced out of one side of its rod- or bean-shaped cell. It appears to be a very cunning little bacterium since it extracts energy out of the iron and manganese and deposits the spent iron and manganese as oxide pellets in its ribbon-like tail. Thus, it produces a super long tail which is discarded, and then the cell can swim off through the water to start all over again. This explains why water can be seen with so many tails and yet very few heads! These bacteria do appear to need oxygen to grow, but very little (even parts per billion) may be enough and they can easily be overdosed when there is too much oxygen present. Because of their long distinctive ribbon-like tail, water is often diagnosed as having principally a *Gallionella* problem when, in practice, the slimy "gloop" commonly seen around them contains far more bacteria which could mean the actual cause of the microbially influenced problems.
2. Sheathed IRB are a large group of IRB that are very specialized and relatively easy to recognize microscopically by their shape and the way they grow. In almost all cases, the bacterial cells grow within a slime tube or envelope where iron and manganese oxides have been deposited to often give the growths an orange to brown or black color. Cells are often able to escape from these tubes or envelopes and swim off into the water to set up new colonies or slimes elsewhere. Some waters are dominated by one or more of these types of IRB, but not enough is known to diagnose why the different forms of dominance occur. Common genera include *Leptothrix*, *Crenothrix*, and *Sphaerotilus*.
3. Heterotrophic IRB group also includes many bacteria which grow or survive in water and will take up iron or manganese into their cell walls or the polymers immediately outside the cells. These bacteria all live on organic materials that are present in dissolved or suspended forms in the water. Some of these bacteria can remain active even when the amounts of the organics are present in parts per million or even parts per billion. These bacteria often play key roles in the degradation of even very toxic and/or carcinogenic chemicals. One could almost visualize these bacteria as being a very important part of Nature's Vanguard in cleaning up the many organic pollution spills that occur from time to time. Their activities are utilized as a part of the bioremediation processes designed to contain and then remediate spill and hazardous waste sites. They commonly grow in biofilms, slimes, nodules, foams, and encrustations in soils, waters, muds, and all types of surfaces. Slimes tend to grow where there is very little oxygen present, but some oxygen may often be essential for growth. If the oxygen is taken out, these iron-rich slime microbes in part pack up their bags and move on to the nearest place where there are low levels of

oxygen present. Being very innovative, some cells can substitute nitrate for oxygen and are still able to respire and grow when oxygen becomes absent from the environment. That means in the absence of oxygen, slime can still form when nitrates are present. This can be a common occurrence where there has been pollution of the groundwater with organic wastes. Remember that in water wells with many slimy growths on the screen, on pumps and in the aquifer, the chemistry of the water would have been changed considerably by these HAB. These growths fall into four major groups based upon their colors (which are interchangeable) which are grey, brown, black, and red with black dominating under oxygen-free (anoxic) conditions while the grey is common where there are very low concentrations of iron or manganese.

2.7 SLIME-FORMING BACTERIA

Why do bacteria produce slimy growths which interfere in many ways with the production performance of a system? The slime consists of a massive amount of long stringy molecules called polymers which hold water and protect the bacterial cells growing in the slime from any toxic agents that may be in the groundwater. These polymeric slimes are super "sponges" and will take up and concentrate much of the nutrients and other possibly useful chemicals from the water and concentrate them into the slime. When a cell dies in the slime, all of its components are fed upon by the neighboring cells so that nothing is lost in this super-efficient albeit cannibalistic community. These bacteria are commonly associated with plugging in water wells and tend to dominate when the plug is still loose and mobile ("snot-like" is the popular term some use to describe this event). Generally, these slimes are not very granular (gritty when rubbed between the thumb and the finger) and are usually relatively easy to attack when treating an infected well. On some occasions, these bacteria can form a major part of the population in biocolloids. Usually, these slimes can be disrupted by a combination of acid and penetrant treatment.

2.8 TOTAL BACTERIA

Like the majority of plants and animals, most bacteria are not dangerous to humans. They occur widely in soils and waters, reaching populations sometimes in the million per gram or per milliliter. They come in all sorts of shapes and sizes and yet each group has somewhat different preferences for habitat, food, and the level or need for oxygen. This makes it very difficult to accurately quantify all of the bacteria in water. Bacteria can make the water cloudy (it can take as many as 100,000–10 million cells per milliliter to do this!), taste peculiar, and generate odd odors. In addition, the total bacterial population can harbor some potentially dangerous organisms to human health. Traditionally, it has been the coliform test that has been used to determine whether there are any dangerous bacteria present in the water

since the coliforms are considered to be the best indicator of fecal pollution (a major health risk consideration).

In general, when looking at the total number of bacteria in water, the idea is normally to determine what level of bacterial presence by population in the water. Activities created by these populations can include corrosion, degradation, putrefaction, plugging, and self-purification. The last function is a phenomenon wherein the waters can undergo a gradual improvement as a direct result of pollutants being degraded or removed from the water (i.e., a natural filter). Oxygen is a major driving force, along with temperature, in helping to speed this process. Because there are so many different functions occurring concurrently as a result of the presence of the various bacterial groups, it is very difficult to monitor all of the groups in one simple test. As a result, a somewhat confusing range of tests has been generated, but predominant amongst the methods are the standard plate count (SPC) and the total plate count (TPC) that have been used as industry standards.

2.8.1 SPC Method

This test is reasonably fast but tends to indicate only the bacteria which grow rapidly at higher temperatures (30°C–40°C) in 2 days (these are common times and temperatures that many laboratories use). These bacteria are more likely to have originated from warm-blooded animals (including humans) rather than from the environment where temperatures are commonly at less than 30°C. The test originally used to be a pour plate technique and is still used sometimes. In this test, the water is diluted and then mixed with molten agar at >45°C so that colonies are mixed into the molten agar before it sets. This means that they can grow throughout the agar and be easily counted. To many bacteria growing naturally in the environment, this exposure to the $45+$°C temperatures can create a heat shock which can be lethal. As a result, many of these shocked bacteria will not grow in 2 days when the counting of the colonies is often done. All colonies are counted and weighted by the dilution factor to give the bacterial population as colony forming units (cfu) per milliliter.

Each colony is presumed to have been formed from one bacterial unit (which can consist of one cell or more than one cell that has formed into a clump, possibly of diverse but compatible types), which grows distinctly within, or upon, the agar. To eliminate the temperature shock effect, another technique can be used whereby the diluted water is streaked across the surface of the agar. The food medium commonly used to grow bacteria for this test is the SPC agar medium which also tends to be too rich in nutrients for many of the environmental bacteria! The TPC was introduced to obtain a better handle on the bacterial population.

2.8.2 TPC Method

There is a much greater flexibility in the way that the spread plate technique can be used. The medium has not been so rigidly laid down and there are seven or eight

different media that are in widespread use. Paralleling this, there is flexibility in the incubation temperatures and times that are used. Common temperatures used include 8°C–10°C, 20°C–22°C, 25°C–28°C, 30°C, 35°C–37°C, and 45°C. The selected incubation temperature relates directly to the incubation times, particularly at the lower end of the scale. Prolonged incubation allows more time for the bacteria to grow. For example, at 8°C–10°C, bacteria can take up to 6 weeks or longer before forming a visible and countable colony and so the plates should be incubated to the longest term. In requesting a TPC, questions should be raised as to the culture medium, temperatures, and times that are going to be used.

One of the frustrations in requesting bacterial population counts of a water sample is the long delay before the numbers are generated, perhaps long after the treatments should have been, or was, implemented. A number of new techniques are being examined to cut down this response time. These include particle counting, epiflorescent microscopy, sophisticated staining, and biochemical methods which search individual enzymes, genomes, or marker chemicals.

2.9 MICROORGANISMS IN WATER WELLS

Much if the preoccupation of biofouling in water wells has focused on the bacteria. This is because they dominate at the redox front around the well, partly because some of the bacteria have adapted or are adaptable to conditions that are reductive (oxygen-free). Those microbes that are dependent upon oxygen for growth and survival (such as the protozoa and the molds or fungi) cannot survive so well under these conditions of severe oxygen stress. Corrosion, rotten egg odors and black slimes are commonly related to the activities of SRB that include three major genera. Another group of microorganisms that can also be a concern are the viruses. These are not so much living reproducing cells but molecular parasites that can only survive by getting into cells that belong to living species that are forced to do their reproduction for them. Here the virus infects the host cell and induces the cell to reproduce the virus particles, because they (the virus particles) cannot do it for themselves. Each of these groups of microorganisms has a very different potential role to play in water wells and these roles are discussed in the next sections.

2.10 ROLE OF MOLDS (FUNGI) IN WATER WELLS

Molds are combined into a group of microbes that all require oxygen to survive and break down organic material as their major energy source. They are simple-celled creatures that grow in long (sometimes branching) threads that can penetrate deeply into porous media provided that oxygen is available. A growth of molds looks very much like a fluffy patch of wool with threads going in all directions. Most molds reproduce by either the threads (called mycelia) breaking up and continuing to grow, or by the production of small, often spherical, cells called spores. These spores are like seeds are to plants, although very much smaller. They are small neutral pods

within which the fungal cell is totally at rest (suspended animation). When one of these spores comes into an environment that would be conducive for colonization then these spores come back to life and begin to grow. Fungi produce millions of spores in order to ensure survival. If you look at a mushroom (one of the most sophisticated of the fungal groups), the gills inside the mushroom are just one mass of packed spores ready to be released and attempt to colonize new environments. Each mushroom produces millions upon millions of spores and yet all of the mushrooms in a mushroom patch are only 5% of the total biomass, the rest is mycelium down under the ground preparing to grow even more mushrooms to produce more spores. Toadstools are even more sophisticated and some will actually puff spores out into the atmosphere.

Because the molds have some very specific requirements such as oxygen and a semi-saturated environment, they tend to be found in locations at the static water levels in the formation. Commonly if the water level does not move much then the molds will build matt-like lateral structures all along the top at, and above, the water level in the semi-saturated porous media. These microbes are able to degrade a variety of organics and tolerate more acidic conditions than most bacteria. Furthermore, many molds are able to produce antibiotics to limit competition from other microbial species including bacteria. One of the most well-known antibiotics made by molds is penicillin.

In most wells, the molds cannot compete very effectively under the conditions of water saturation and oxygen limitations. However, there are occasions when the mold spores can have a significant presence in the well water. One occasion for getting a high number of mold spores in the well water is when the groundwater is under the direct influence of surface waters. Here it can be expected that the surface water, in penetrating down into the aquifer, passes through the static water level in which there are large lateral mats of mold growth. As the percolating surface water passes through these mats then large numbers of mold spores can become detached from the mycelia and enter the water flow. Additionally, and particularly for the shallower wells with a low static water level in the well, there is the possibility that lateral mold mats are growing at, and slightly above, the water level around the bore hole. If the well is being pumped on a regular basis then these molds may grow through the cone of depression created by the pumping action. That would mean there would be a potential for more intense mold growth (with higher mold spore populations) in that "smear" growth zone created by the pumping action. If this is happening there might be occasional releases of mold spores into the well water that could be associated with the pumping activity.

Molds do not normally get involved as major "players" in the plugging of water wells as such. In the laboratory, experiments have found that molds, once they have formed a mat can significantly effect water movement through porous media principally because of the nature of the mats that are grown by the fungi. Generally such mats sit along the oxidative side of the redox front but only if the conditions are semi-saturated. In most wells the conditions are saturated with water and semi-saturated oxidative conditions are only found much further up the bore holes towards the grade.

2.11 ROLE OF PROTOZOA IN WATER WELLS

Protozoa is the name given to single-celled animals. They are considered animals because they are all aerobic (require oxygen) and commonly have some function for taking organic material inside the cell for digestion. In fungi and bacteria, the digestion takes place outside of the cell with the useful products then being taken up by the cell. In general, the protozoa are larger and more sophisticated than other microbes even having flexible cells, complex propulsion mechanisms (sometimes), and more refined reproductive cycles. Many protozoa can live in organic-rich particulate material and require significantly larger amounts of organic materials (since their life processes demand more energy use).

Protozoa can thrive in water wells if there is a lot of available oxygen and an abundance of organic particles upon which the protozoa could feed. In some shallow and wide bore wells, these protozoa can become the "Kings of the Heap" feeding off the other microbes that may have been growing down the hole. This changes, however, if there is a high-iron concentration in the well and, more particularly, in the biomass growths. As the amount of iron in the biomass increases from 7 to 20% then the protozoa feeding on the slimes find this additional iron prevents them from feeding. Thus it is much less common to find a shallow well with a high-iron content supporting a rich and diverse protozoal population when compared with a similar well that had low-iron content (e.g., $<1\%$ in the biomass and <0.1 ppm Fe in the water). It is interesting to note that for the IRB, the iron protects them from being eaten alive by the protozoa while other microbes not protected by the iron shield can rapidly fall prey to the protozoa.

Two protozoa of concern in water wells are *Giardia* and *Cryptosporidium*. These two genera rank as two of the major causes of gastro-intestinal infections in humans. *Giardia* has had a long history in microbiological terms because it was first described by Leeuwenhoek in 1681 when he was using his single lens microscope to examine his own stools. The genus was not named until 1882 (almost exactly 200 years later) as *Giardia*. From as early as 1850, organisms resembling the (now classical) shape and size of *Giardia* were being reported in a variety of animals from cats, and dogs, to rabbits and hamsters, as well as in humans. For humans the common name is *Giardia lamblia*. There are two phases in the life cycle of *Giardia* species. There is an active phase in which the cells are called trophozoites, and a passive phase (resting stage) called a cyst. It is in the form of cysts that *Giardia* species are able to travel from one host (commonly from dispersed stool material) to another host (infection being via food, water, or the anal–oral route). There is a wide variety of symptoms when an acute infection occurs with patients commonly exhibiting diarrhea, flatulence, foul stools, cramps, anorexia, and nausea. Generally, patients will have cysts in the stool and transmission could occur if greater than 25 cysts were consumed.

Water wells were responsible for 67% of all of the waterborne outbreaks reported to the Centers for Disease Control (CDC) over the period from 1991 to 1998 but caused only 34% of the cases. This would indicate that the outbreaks from wells generally impacted fewer individuals (cases) than other water sources. *Giardia* species were found to have been the cause of 38% of the outbreaks and

caused 22% of the cases meaning that each outbreak generated a smaller case load than other pathogens might do. Infection would mostly have stemmed from the cysts that are able to survive in the water for a much longer time than the trophozoites. For the cysts to have been involved in an infection from a water well source, there are two critical factors

1. Cysts are in a resting stage and would not be able to grow within the wells environment and
2. Source of the cysts in the well is most likely to have been because the groundwater was under the direct influence from surface waters that were contaminated with the *Giardia* cysts.

Another protozoan that can have a major impact through waterborne infections is *Cryptosporidium*. This genus has been associated in 20% of the waterborne outbreaks but generated a greater number of cases (58%) than *Giardia*. This would suggest that *Giardia*-sourced infections are the more common of the two protozoal pathogens, but *Cryptosporidium* is the more serious infective agent, generating 58% of the cases, not including the outbreak in Milwaukee in 1993 where there were 403,000 cases involved in the single outbreak.

Cryptosporidium species differ from *Giardia* species in the following ways

1. Symptoms of infections in humans is the generation of watery stools (for 3–10 days) accompanied by nausea, vomiting, abdominal cramping, low grade fevers, and headaches.
2. Fluid loss can be severe ranging commonly between 3 and 6 L per day but in some it can reach as much as 17 L.
3. Transmission is commonly by oocysts and cross-infections can occur through the feces of farm animals, mice, birds, and other animals.

Human-to-human transmission can occur directly or though indirect contact with contaminated feces or respiratory secretions. For the water well to be the source of the initial infection would mean that oocysts had entered the groundwater through the direct influence of surface waters which were carrying these cysts. The potential for human-to-human transmission appears to be much higher than for *Giardia* and this would explain why the number of cases per outbreak is much higher. This would be the case for the outbreak in Milwaukee. For both of these protozoa, transmission is through a cyst that is able to survive for some time, but not reproduce, within the water well environment.

2.12 ROLE OF VIRUSES IN WATER WELLS

Viruses were thought, in the late part of the nineteenth century, to be very small bacteria that could pass through a porcelain filter and just could not be grown. It was found at the very end of that century that this hypothesis was not true and they were unique organisms that were not really alive but were parasitic upon other living

cells. Today viruses are defined as being subcellular agent each consisting of a core of nucleic acid surrounded by a coat of proteins. These agents have to parasitize other cells in order to reproduce. They are not capable of reproducing without invading a specific type of cell and then usurping the functioning of that cell to replicate the virus particles that are released when the cell disintegrates. Today it is well known that there are animal viruses, plant viruses, and even viruses that will attack bacteria (bacteriophage). Since the viruses contain only parts of a cell, they are very small, generally in the range of 0.025–0.3 μm. This is much smaller than bacteria which are commonly anywhere from 0.5 to 10 μm in size. In order for the virus particle to succeed, it has to take over a vulnerable cell and that particle has to be able to enter the host cell through the cell wall. Once inside the cell, the particle has to take over the cells ability to replicate in order to replicate virus particles rather than the normal parts of the cell itself. A single cell may, once infested, yield hundreds of virus particles generated from the original virus particle.

Commonly virus particles are vulnerable in the environment because they appear to have no defence mechanism to prevent accumulation and destruction within biomass associated, for example, with water. From the CDC records for waterborne infections from 1986 to 1999, it has been found that viruses were implicated as the cause in 4% of the outbreaks and yielded only 2% of the cases. Significant detections occurred in 1986, 1987, 1988, 1989, and the last one was in 1995. The first four incidents were considered to be the Norwalk strains while the 1995 incident remained unidentified.

Human enteric viruses can contaminate municipal drinking-water wells, but few studies have examined the routes by which viruses enter these wells. It is believed that flooding with waters carrying the viruses could cause a significant entrance of viruses into the well. There have been numerous reports of human pathogenic viruses in groundwater from shallow sandstone, and sand and gravel aquifers. Many public water systems draw water from confined aquifers that are considered protected from microbial contamination by overlying groundwaters.

Enteroviruses have been detected in well waters. Possible routes of transport for these viruses include windows in the aquitard due to heterogeneity in the thickness and/or lithology of the siltstone/shale interfaces. Flaws in well construction, such as fractures or bridges within the grout seal set between the casing and borehole annulus may also provide a pathway for rapid migration across the aquitard.

A clear concern relates the ability of such small particles, dependent upon the parasitism of specific types of cells as the only means of reproduction, to survive in the relatively hostile environment of water wells or surrounding aquifers. Viruses have a small size and are coated in proteins and sometimes fats. Such a small organic particle would stand a high probability of becoming accumulated in the biocolloids and attached biomass within the porous media and the surfaces in the well. Once bioaccumulated, the virus particle is most likely to be degraded by the intrinsic microorganisms in the biomass. Here the virus particle would become an attractive source of proteins, nucleic acids, and possibly lipids. There is no known defence mechanism recognized today that

virus particles can use to protect themselves from such forms of entrapment and degradation. Under reductive conditions deeper inside the groundwater systems where there is a low level of biological activity, it could be expected that the virus particles may survive for longer periods. In the modeling of viral movements through groundwater systems, it is very important to include the interfaces that would be generated between the virus particles and the biocolloids and biomass in the groundwater through which the particles are passing. This area of science has yet to be fully explored from the general microbial perspective.

2.13 ACUTE GASTROINTESTINAL ILLNESS OF UNKNOWN ETIOLOGY

It is significant that there remains a large number of waterborne infections that go undiagnosed. According to the CDC waterborne infection database for the period from 1991 to 1998, 38% of the outbreaks and 58% of the cases fell into this category as acute gastrointestinal illness (AGI) of unknown etiology. There are many challenges created by this large percentage of undetermined gastrointestinal infections. One main reason for the large percentage of AGI is the fact that diagnosis requires a fully functioning, comprehensive microbiology laboratory that is able to effectively isolate the pathogen responsible for the gastrointestinal (enteric) infections.

Canada has a well-established surveillance for enteric disease which is limited by the passive nature of surveillance data. Consequently, there are significant gaps in information describing the magnitude of enteric disease in the population at large. To have a fully effective surveillance system, an individual must first be available for a medical care provider to ensure submission of a specimen for microbiological testing. Even this process only captures a fraction of enteric disease episodes occurring in the community. Although underreporting is well recognized by the public health community, the lack of any quantification frustrates attempts to make accurate population level estimates from existing surveillance data. One of the side effects of this would be that a significant number of illnesses become diagnosed as being of unknown etiology.

In Canada, for example, there was a survey administered to 470 microbiology laboratories across Canada of which 408 (87%) responded. This study identified a number of inter-laboratory and inter-provincial/territorial variations in criteria for testing stool specimens. A small number (3%) of specimens were rejected because no transport media was used, the stool was fully formed, or the container was damaged or contaminated. Routine testing for *Salmonella*, *Shigella*, *Campylobacter*, *E. coli*, and *Yersinia* was common with 100, 99, 97, 96, and 95%, respectively, of laboratories routinely testing for these bacteria. Other pathogens, such as *Plesiomonas* and *Vibrio*, were routinely tested by fewer laboratories (54 and 38%, respectively). This would mean that some AGI reflected a failure to diagnose the pathogen because it was not included in the routine surveillance.

Differences in laboratory policies were observed regarding:

1. Testing of repeat specimens.
2. Testing specimens from inpatients who have been hospitalized over a certain length of time.
3. Testing fully formed stool specimens.
4. Testing stool received without transport media were noted.

When comparing the effect of these policies on the likelihood of identifying enteric pathogens, there were few statistically significant relationships. Overall, participating laboratories tested (culture and/or molecular methods, including toxin detection), 459,982 stool specimens for enteric bacterial pathogens (excluding *Clostridium difficile*) in the year 2000. In comparison, 392,023 stool specimens were examined for enteric parasites, 177,696 for *C. difficile*, and 14,051 for enteric viruses. Of the laboratories testing for viruses, 74% indicated never testing for astrovirus and 69% never testing for small round structured viruses, caliciviruses, Norwalk or Norwalk-like viruses. On average, 5.0, 7.6, 15.3, and 18.9% of stool specimens which were tested for bacteria, parasites, *C. difficile*, and viruses, respectively, were positive. The overall proportion of tests positive for a bacterial, parasitic, or viral pathogen was 8.8% (the sum of all positive isolations divided by the sum of all specimens tested for bacteria, parasites, and viruses). This illustrates that the AGI is at least in part a reflection of the ability of the laboratory to test the stool sample comprehensively. If water had to be tested for these enteric pathogens then the probability of a positive detection would be narrower simply because the pathogens would be more dispersed in the water than they would be in the stool. Stool sampling and testing proved to be much more effective for patients admitted with AGI. American and European surveys tended to find that between 1 and 6% were positive for enteric bacteria. Higher detection rates have also been recorded at between 16 and 19% in the Netherlands and the U.K., respectively, with much higher detection rates with stool enhancement.

3 Sampling Procedures

3.1 INTRODUCTION

There are clients who spend thousands of dollars on getting the most sophisticated and expensive testing performed on a water sample, which may not actually represent the critical conditions that are occurring at the sampled site. Generally, considerable thought has to be given in sampling the water well since here is Pandora's Box that is created by the question: "what is it that needs to be sampled?" Most of the biomass may be attached to a surface which means that the water flow may not reflect this biomass at all unless the biomass is detaching and moving into the water. If the biomass does detach, then the biology and chemistry of the sampled water will change dramatically. If the well has just been powered up, then there would be turbulence in the well, which will bring up the deposits that have collected at the bottom of the borehole. These would swirl up and become a part of the sample. Such a sample would contain a lot of particulates, causing false chemistry and would tell nothing about the quality of the water itself.

Because the water well has natural filters that are composed of several cylinders of biomass, there is a major challenge as to when to take the sample. If the well is pumped for a considerable time, then gradually the water starts to resemble the groundwater beyond these biomass filters. Care has to be taken to decide when to take the water sample and also what information is needed from that sample. These range from the basic health risk concern (are there any coliform bacteria in the well?) to the production concerns (is the well beginning to plug and fail?) and water quality issues (are undesirable chemicals beginning to break through the biomass filter and appear in the water?). Perhaps the very first thought has to be for what purpose is this sample being taken or do we need to take a series of samples?

3.2 SAMPLING WATER WELLS

Sampling has no value unless there is some form of subsequent testing even if it is limited to smelling the sample and judging its clarity and color. Sampling protocols control the value and precision of the testing that may be subsequently performed on that sample. Testing is the act of following some established (certified) standard procedures in order to generate data that have a perceived value almost regardless of the manner in which the sample(s) was (were) taken.

The water well presents a number of particular challenges because the wells have natural filters with many of the chemicals and microbial cells moving in and out of these filters. Since these filters are out of sight, it is easy to put them out of mind. Even monitoring wells and piezometers have these natural filters that can impact on the sample data and possibly compromise the value of the interpreted data. When sampling from such "dormant" sources as the monitoring wells and piezometers, it has to be recognized that there is microbial activity around these wells that could affect the data being gathered. Consider the cost that is expended on taking the sample and compare that to the cost of testing the sample afterward. Often 10–100 times as much can be spent on the testing than on the cost of getting the sample.

Another concern relates to what happens to that sample from the moment it has been taken to that moment when the test is performed? If those tests are performed in a laboratory, then there is a time period during which the sample is being moved from the sampling site to that laboratory where it then joins the queue. There are two to four events that can occur during this transport and affect the quality of the data being generated. There are a number of steps that need to be considered in the decision to conduct a sampling program. These are discussed below based upon the precise objective of the sampling and subsequent testing. Unlike surface waters, groundwaters are commonly flowing slowly through voids or fractures until trapped by the well and pumped out. In moving faster and faster with increasing turbulence toward the well, the groundwater is therefore moving through the channels of least resistance to flow. That resistance to flow is at least partly the result of the dispersed biomass growing within the voids and fractures, particularly in the natural filters immediately around the well. There is an interaction between the groundwater and this biomass that can reduce or divert flow, causing some chemicals to be removed from the water to accumulate or be degraded in the biomass, and cause some biomass to be released from these attached biomass growths and enter the groundwater. Once the groundwater is within the borehole, there are two possible interactions. If pumping is active, then the turbulent forces continue to mount and the water pumped out of the well. If the pumping is not happening (passive), then flow is minimal. Under these circumstances, there would be little flow inside the well and a tendency for the heavier materials (such as iron-rich biocolloids) to separate and sink down the bottom of the borehole. It is common for the bottom of the well to collect these materials along with any sands and clays that may have entered. All of these then collects as a thick silty mass that builds up unless it is disturbed by excessive pumping action or very low water levels in the well. It can be expected that when a well is powered up, the physical forces created by the water changing from passive almost no-flow situations to high flow will create a number of conditions:

1. Biocolloids floating in the water column, which are affected by the pumping action, will be drawn out of the well creating high microbial populations in the pumped water.
2. Turbulence could disturb the settled silty mass in the base of the borehole causing silts and dense biocolloids to be released into the pumped water.

3. Physical forces involved in the pumping could cause some of the slimes, encrustations, and other growths to peel away from the walls. The denser of these materials may fall to the bottom, while the lighter fractions would be pumped out in the water.
4. Slots, perforations, or fractures closer to the zone through which the groundwater is being actively drawn into the well could release growths that have been forming around these "entrances" into the well. This could also contribute to that material present in the water at the first of the pumping sequence.
5. Groundwater moving closer to the well will be accelerating and under turbulence while it is moving through the natural filters around the well. This would create variations in the water quality depending upon the amount of sloughing (sheering) from the biomass that is getting into the groundwater.
6. Groundwater moving in from the formation aquifer reflects the background chemistry, biology, and physical characteristics of the formation water.

There are therefore six stages in acquiring the groundwater through pumping the well, which can affect the quality of that water.

The act of taking a sample has to take into account the shifting that will occur in water quality, which will most likely happen during the early phases of pumping the well. It is therefore important to determine the most suitable time for taking one or more samples from a well. If one sample is to be taken, then this might be for routine monitoring of the well for regulatory and maintenance purposes. If more than one sample is taken in sequence, then that will determine in a more specific manner any potential problems in the well. It should be noted that the manner of taking the sample and subsequent care of the sample for many test procedures is addressed in the Standard Methods for Water and Waste Water and will not be addressed further here. This chapter will be limited to the timing for taking water sample(s) to meet the needs for the determination of the events being monitored. If a single sample is to be taken, there would normally be a need to ensure that it reflects the normal pumped water from the well without the impacts of the events described above relating to stages one to six when there are significant likely impacts occurring between the pumped water and the natural filter and the borehole. This will be addressed separately from taking a series of samples which will be able to give more precise information on the status of the natural filters and the borehole from the biological, chemical, and physical perspectives.

3.3 SINGLE SAMPLING PROCEDURE FOR WATER WELL

If a single sample is to be taken from the well, then a prime driver in the decision process would be the purpose for taking the sample. This purpose could materially affect the timing of the pumping of the well that the sample would be taken. Ideally, that sample should be taken from the well head or as close to the well head as

possible. In practice, particularly for smaller noncommunity wells, the sample site is often the kitchen faucet and the water would have been subjected to all of the filters, conditioners, treatment devices, as well as storage tanks and pipes that connect the faucet to the well. All of these sites can cause changes to the biological characteristics of the water sampled (Table 3.1). For community wells that are often connected to water distribution systems including treatment and storage facilities, there may be sampling ports within the treatment plant relating to each well or a set of producing wells. There is always a possibility of additional biofouling affecting the integrity of the sample and it is a good idea to select the sampling port that is closest to the well head for the specific well being tested and with the minimum of pipe, interconnections, storage, and treatment plants.

Precision in the test data is therefore likely to improve with the longer delay times before sampling after starting up the production (pumping) of the well. If only a single sample is going to be taken, then the time delay from the starting of a pumping sequence becomes critical to the purpose of the testing. This means that the time into pumping will impact on the characteristics of the water sample.

Having decided on the site for taking the sample, the next decision has to be precisely when to take that sample. For example, a water sample taken as the pumping begins or even 5 min into the pumping sequence is likely to reflect the impact of biological and chemical detritus that had collected and been sheered off from the slots, perforations, or fractures into the water. Water samples taken 10 min into pumping are now likely to be more affected biologically by any sheering of biomass from the porous media close to the well. Waiting 20 min into pumping for taking the sample is likely to now resemble the groundwater that has come into the sphere of influence of the well from the aquifer, but there may still be some sheering (sloughing) from the biomass that could affect the water quality. As the pumping continues, it would be expected that the conditions would stabilize and the quality of the water would become more of a reflection of the groundwater entering the sphere of influence created by the operation and less of a refection of the interactions between the entrancing water with the biomass surrounding the well in the natural filters.

If the objective is to determine the in-well fouling, then a 0- and 5-min sample should both be taken and compared. If the objective is to determine the health risk factor through undertaking a total coliform bacteria test, then 20 min may be a more suitable time. This is because there is a greater probability of total coliform bacteria being normally present in the detritus, the biocolloids, and the natural filter close to the borehole. Even at 20 min there is a greater potential for recovering total coliform bacteria than at 120 min or when the well is pumping for longer than 240 min. One problem is that often the sample time is not seen as important and the sampler simply just wants to take the sample and get it off to the laboratory. Waiting 20 min after the start-up of the pump now becomes a nuisance even though this is still a very minimal time. One hundred twenty minutes is an ideal realistic time for this purpose.

While the time from turning on the pump to the taking of the sample is important, equally important is the length of time the pumps were off before this

TABLE 3.1
Effect of Time After the Start of Pumping on the Likely Biological Characteristics of the Sample

Time (min)	Biological Characteristics	Other Salient Comments
0	This would reflect the disturbed detritus from the base of the borehole and is highly likely to give higher biological activity. Greater potential for total coliform bacteria to be recovered. Also, the high biocolloidal contents may also support larger and more active bacterial numbers	Water sample more likely to have a high total suspended solids (TSS) and turbidity. There may also be higher metal contents associated with the detritus brought into the sample
5	Water sample would contain biological material from the sloughing biomass coming from the slots, perforations, or fractures and may still contain biocolloids from the borehole itself. Bacterial populations would be reflecting the activity within the borehole and in the immediate environment of the borehole	Water quality would have a raised TSS reflecting the degree of sloughing that has occurred from the immediate environment around the well. Duplicate samples are likely to show some variations in the chemistry
10	Water sample has come from the zones within the natural filter and would reflect the organisms that were either in the voids or sheered off from the biomass through which the water was passing. Bacterial populations would therefore still be high depending upon the amount of sloughing that was occurring at the moment when this water was passing through the biomass	TSS should be much lower in this sample than in samples taken at 0 and 5 min unless the well is very badly biofouled. Duplicate samples would still show variations in the chemistry resulting from sloughing
20	Normally the flushing actions associated with starting up of the pumps would be coming to an end and the biological characteristics of the water would be more reflective of the "normal" water quality coming from the formation	TSS should now be lower and relatively stable except when an infrequent major sloughing event has occurred. Duplicate samples should show similar chemical and physical characteristics
120	In general, water sampled at this time would be dominated by the characteristics of the formation groundwater from the aquifer. It would however be influenced by any routine additions associated with the biomass in and around the well	Chemical characteristics should now be stable and the TSS constantly low. Duplicate samples should yield comparable data that show precision
Continuous	Should reflect normal biological characteristics	Should reflect reasonably constant chemistry

Note: Time indicates the time into a pumping sequence at which the sample is taken. Continuous refers to a condition where the well is being pumped continuous or the well is being operated greater than 70% of each daily period and the sample is taken greater than 240 min into the cycle.

sequence was even started! The downtime (length of time the pump is off before pumping) can be a critical factor. When a pump is off then the water flowing through the borehole is reduced to the same flow rate as within the formation (background). Like all animals and plants, the biomass in and around a water well gets used to a routine and adapts to that. Remember that this biomass is a living amalgam of biology and chemistry, which is interfering with the physics of water flow. While the well is functioning in a routine manner (e.g., 70% on and 30% off in any 24-h period), the microbes within the biomass adapt to those conditions.

Changing conditions in water wells can cause the microorganisms to go into shock. Ideally, this change should be radical (e.g., turning a well off for 7 days) to cause repercussions within the microbial community in the well. Generally what happens here is that oxygen becomes a premium need and the redox (oxidation–reduction potential, ORP) will tend to decline to more reductive conditions. The lack of flow (bringing nutrients) and changing ORP means that many of the microorganisms can go into shock. That shock now dictates that they try to migrate to some more suitable environment where they can again become active and grow. The microbes have no idea where their heaven would be but they do start the migration process! This process begins with the microbes within the affected biomass moving from the attached biofilms (slimes) up into the groundwater. The objective, from the microorganisms' perspective, is to now move through the groundwater until a more suitable environment occurs. Thus, during the first 7 days of the well being shut down, there is a mad panic in the biomass and many microbes now move into the groundwater. When the well is pumped again, these microbes can be detected in the pumped water. If the aim of sampling is to detect whether a well is biofouled and supporting a large community of different microbes, then this is a way to detect them. In shutting the well off for 7 days followed by immediate sampling, a far greater ability now exists to detect the microbes normally growing, and active, within the biomass. Techniques to develop this and generate zones of interrogation projection (ZIP) are based on this premise that turning off the well for a long period of time will improve the potential for recovering microbes in the sample(s) when it (they) is taken.

Taking a single sample from the water well does not have the ability to give a great deal of information about the well. It is through the careful sampling during a pumping sequence that can provide this information. This is the subject of the next section in this chapter. Clearly the selection of the time and place for taking a sample becomes critical to the value of the data generated when the sample is finally tested. For example, if the objective is to determine whether there are total coliform bacteria in the well, then the probability of detecting these goes down as the well is pumped. Samples taken right at the start of pumping are likely to give a much higher level of positive detections for total coliform bacteria simply because many of the total coliform bacteria can form a part of the natural biomass that is in and around the well. If there is major risk of coliform bacteria causing a health risk from a well, then these coliform bacteria should also be detected in samples taken later into the pumping cycle when the characteristics reflect more the background levels of coliforms within the well's sphere of influence.

3.4 MULTIPLE SAMPLING FROM WATER WELL

The decision to take more than one sample from a well has to be accompanied by a good explanation to the client since there would now be more expenses involved in the testing and then the interpretation of the data. Multiple sampling means taking a timed series of samples to determine whether some events might be more dominant (evident) in some of the samples than others. This is different from duplicate sampling where a single sample is taken and then tests are done in duplicate or even triplicate to confirm that the data do generate confidence. In some testing, it is common for the occasional testing of a given sample to be out of line (outlier) with the other test data. Multiple sampling refers to the fact that a sequence of samples is taken and there remains the option to perform single or duplicate tests on the sample.

Multiple sampling is much more of a forensic tool to determine the state of fouling in the well. Most commonly the state of the well of most interest relates to the level of biofouling that is influencing the well's production; and also the effectiveness of any treatment applied to reduce some aspect of fouling that is affecting the well. There are a number of standard strategies that can be used based upon the same principles as a pump test. In the introduction to this chapter on sampling, it was described how the groundwater quality is affected as the water passes through the various filter zones around the borehole. Initially the water is basically the borehole water column that has been agitated (stirred up) by the start of the pump extracting water from the well. Water now moves toward the borehole and the major impact is with the biomass and materials immediately around the well and in the slots or perforations. Following this, water entering into the well comes from zones where the natural biomass filters are situated. This would then reflect the characteristics of the biomass to a greater or lesser extent depending upon the state of the growth. After this water has been extracted, the next water comes from the edge of the sphere of influence of the well and more closely resembles the characteristics of the aquifer. There would be variations that relate to that amount of material that would be entering or leaving the groundwater as it flows toward the well. Following this, provided that there has been an efficient flushing of the natural filters and the borehole, the produced groundwater now being pumped from the well is likely to closely resemble the water in the aquifer itself outside of the sphere of influence of the well. In conducting a sequence of sampling from the same well, it is possible, by testing these samples, to determine the scale of biological and chemical events that are interfering with the physical movement of water into the well.

In deciding the sampling times during a pump test for the well, there are a number of decisions that have to be made with respect to the well's physical characteristics such as the size of the pump, nature of the formations around the well, and the position of the static water level around the well. These factors mean that it can become a relative nightmare to calculate the position of various biological events around the well in mathematical terms with a reasonable certainty of accuracy. In practice, there are two levels of multiple sampling. At the first level, the timing for the pumped sampling follows standards without concern for the flow rate of the pump. Here only comparative interpretations are possible based upon the

biological and chemical testing of the sequenced samples and the calculated volume of extracted water that was generated during the sampling procedure. For the second level of multiple sampling, physical characteristics of the formation material around the well within the sphere of influence are taken into account. By bringing into account the well volume beneath the static water level and the volumes of the various voids and fractures around the borehole, it now becomes possible to calculate mathematically the mean position for the different biological sites around the well. This assumes a laminar flow along the producing zone within the borehole. If there is no laminar flow, then only a best estimate of the average position of the biofouling becomes possible. One problem with both of these levels is that a simple assumption is made that the biomass within the voids and fractures does not occupy a volume. In reality the biomass along with other accumulates do occupy voids with consequent severe restrictions to flow occurring when 40%–60% of the void volume is now occupied by biomass (plugging) or solids that have become perched within the voids (clogging). If there were either significant plugging or clogging ($>5\%$ of the void and/or fractures occupied by biomass and/or deposits), then automatically the calculations for the position of the events outside of the borehole would be incorrect. The actual difference would be even larger than the calculated positions. Furthermore, a nonlaminar flow into the boreholes would mean that a third dimension would come into play in the projection of the positions for the various observed activities. It is therefore most convenient to limit the extrapolations from the data to the time the various samples were taken and activities and events recorded to volumes based on simple pumped flow rate. Any repeated multiple sampling using the same well and test protocols would yield data that could be compared to the original. Questions such as "has the well become more biofouled?," "where is the biofouling," and "did the treatment of the well after the last testing improve the well?" can be addressed in a comparative manner.

Multiple sampling can allow the zones around the borehole to be established. One such technique determines the ZIP based on the pump times to each of the samples taken. This technique actually allows the position of the different bacterial components in the biomass forming the natural filters in and around the well being tested. Generally speaking it is the iron-related bacteria (IRB) that are commonly found closest to the well with the sulfate-reducing bacteria (SRB) on the outer edges. In between these groups are the slime formers (SLYM) that tend to be close to or overlapping with the IRB and the heterotrophic (HAB) bacteria. Commonly this interface is also where the redox front (shift in ORP from reductive upstream to oxidative downstream) is located. Heterotrophic bacteria can pinpoint this since they will tend to give down reaction (DO) reactions on the reductive side of the front and up reaction on the oxidative side. If SRB get involved with the HAB, then commonly the black top (BT) reaction will dominate, while back in the reductive formation black bottom (BB) will be dominant. If there is a considerable production of biomass that is likely to cause a plugging event, then dense gel reactions may be observed at those sites. For the IRB, this would be in the form of a brown gel (BG) or brown ring (BR) and for the SLYM it would as a dense gel reaction. In the event that groundwater is under the influence of surface waters that contain a high organic loading (such as septic waste or feedlot runoff), there is likely to be significant

organic nitrogenous materials that would be degrading reductively with the production of ammonium. At the redox front there would be nitrification oxidizing of the ammonium to nitrate. When the oxidized water returns to a more reductive environment, denitrifying bacteria (DN) would reduce the nitrate via nitrite to a terminal state as dinitrogen gas. If this is happening in the groundwater under the influence of this type of surface water, then DN is likely to be present and generate the foam reaction in the DN-biological activity reaction tests (DN-BART). If the ZIP shows the presence of DN particularly in one section of natural filters, then it is possible that this is the site at which some nitrate is moving into the well and being reduced to nitrite and dinitrogen gas. Another signal that there could be a situation that the groundwater is under the influence of surface water would be the presence of enteric bacteria. These bacteria are dominant in septic waste and raw domestic sewage. When they are present in the water sample being tested, there is likely to be a terminal reaction in both the IRB- and SLYM-BART giving a black liquid (BL) reaction. This would indicate that there is a higher possibility that there has been an impact from septic waste, animal feed lots, and/or sewage. A BL reaction should be treated as a warning sign that there may be a health risk associable with the well and total and fecal coliform testing should be performed.

3.5 ZIP TESTING

In performing a ZIP using the BART-SOFT version 5, it is possible to gain a better understanding of the biological nature of the natural filters, the position of the redox front, and the location of the various bacteria communities located within the well (Table 3.2). In gaining an understanding of these relationships, it now becomes possible to gain a better appreciation of any plugging that may be occurring around the borehole. Note that if ZIP tests are performed before and after a biofouled (plugging) well has been successfully treated by either some form of preventative servicing or regeneration procedure, then it becomes possible through comparing the before and after ZIP to determine how effective the treatment has been. It should be remembered that any effective treatment has a number of impacts of the surviving microbial communities around the well (e.g., death, trauma, and dispersion). After treatment it can be expected that the water pumped from the well is likely to contain traumatized and dispersed microbes that may still be very much alive and active. If sampling for a ZIP is conducted after the treatment, these effects have to be taken into consideration. Here there would be very high bacterial activity levels recorded simply because of all of the displaced microbes being flushed from the well. Zones of interrogation projection sampling posttreatment wells should not be attempted until at least 6 weeks (42 days) after the end of the treatment. Ideally, it has been found that better precision is obtained at 8 weeks (56 days) or later when the wells has now returned to its normal production cycle.

All sampling and testing of wells have a lesser value if attempts are not made to ensure that there is an accurate measure of the microbial activity. There is a need to maximize the potential for recording microbial activities within and around the borehole.

TABLE 3.2
Recommended ZIP Sampling Times Using BART-SOFT (Version 5)

Number	Time (min)	Comments
1	0	This sample is likely to contain detritus and variable microbial loadings. Its value is therefore limited by the variations that will occur
2	10[a]	An essential sample since it indicates the type of microbial activity that is likely to be occurring around the slots, perforations, or fractures along with the formation material close to the borehole
3	30[a]	This sample will be dominated by the microbes in the middle of the natural filters (biomass) and indicate the possible position of the redox front
4	60[a]	Here the sample would have been taken from the outer edge of the biomass and partially reflect the microbial loadings within the groundwater itself
5	90	Optional sample that may indicate the outer edge of the biomass and the position of the redox front
6	120[a] (2)	This sample should now be from beyond the biomass but would have passed through and so may be carrying some of the microbes released from the biomass during shutdown
7	1440 (12)	After 1 day it would be expected that the well's production would now be dominated by the normal flow of microorganisms through the well
8	2880 (24)	Additional day of pumping means that the shutdown impacts are now 2 days old and the sample is more reflective of the natural flows of microbes through the well

[a] Recommended as minimum sampling times to achieve a useful ZIP. Numbers in parentheses are the time in hours.

In the normal biomass growth cycle down in a well, there are times when all of the microbes may be tightly attached (compressed phase) and other times when the biomass will be expanding (expansion phase) and still a third time when the biomass will be sloughing (collapsing phase, throwing off biocolloids/slimes/"snot balls" into the water). Sampling during a compression phase is likely to grossly underestimate the microbial populations in the biomass. Sampling during the expansion phase will also underestimate since the microorganisms are all busy growing more biomass and are less likely to detach and be present in the water sample. If a sample is taken during the collapsing phase, then there are likely to be a greater number of microbes in the water and the testing would indicate a greater population. To level this playing field so that ZIP tests can be performed in a comparable manner, it is important to disrupt the normal harmonic growth pulse

cycles occurring in the biomass around the well. The mandate should be to determine the levels of activity and the types of microbes present and active around the well. This can be achieved commonly by shutting down the well for a sufficient period of time to ensure a common biological response. Here there lies a conflict between the well user and the need to get precision in the testing method. The well operator would not want the well shut down at all (suddenly the water becomes a precious commodity!) and the objective is to shut the well down for long enough to cause trauma and release of microbes from the biomass. Shutting down the well means the loss of production to the well operator but to the microbes it means even more! Once the well is shut down, the environmental conditions can change dramatically as follows:

1. ORP will become more reductive.
2. There would no longer be any forced flow of groundwater into the well meaning a reduction in available nutrients to the biomass.
3. Natural rhythms of the well are disrupted and the microbes have been growing biomass using that rhythm.

It may be surprising to think that microbes, like plants and animals, do modify their behavior as a result of the environmental condition being placed on them, but they do!

The most convenient manner, when there is an active well pumping daily on a regular basis, is to shut the well off for ideally 7 days (1 week). This becomes only a practical possibility when there are a number of wells connected within the field and the loss of one well for a week is not critical to the demands being placed on the well field. Most commonly 7 days is not realistic since the water demand is too great and there is a reliance on water being generated from that well. In these circumstances, the downtime for the well can be shortened to an absolute minimum of 8 h (or overnight) in order to create the trauma and recover the microbial loading in the well to generate a useful ZIP. While a shutdown of 7 days is an ideal and 8 h marginally too short, there are middle of the line terms when the well may be shut down for 1 or 2 days. With the shorter shutdowns, it can be expected that there will be less precision in the ZIP commonly generated using IRB-, SRB-, SLYM-, HAB-, and DN-BART testers. These time intervals and number of samples to be taken can be adjusted depending upon the nature of the water well being tested. Even if there is a greater than 99% impact on the living biomass, the survivors will grow back in utilizing the freshly cleaned surfaces and the dead organic debris left over from the rehabilitation.

Once the well is capable of routinely pumping but is still shut down (pre-ZIP state), the next challenge relates to the sampling times for the ZIP. While eight possible sampling times are the maximum number of samples to be included in the program, a ZIP can be generated using as few as three timed samples. It is recommended that at least five sampling times are used to ensure precision in the relative location of the bacteria within the biomass. All of these environments are likely to contribute to the types of microbial activity observed at the zero sampling time. In the evaluation of the biofouling status of the well, the zero time

sampling is also likely to contain microorganisms from the drop pipe and lines to the sampling port. This may be a very valuable aid in the determination as to whether these pipes between the pump and the sampling port are being subjected to biofouling.

Interpretations of the BART results do include general evaluations. For example, SRB-BART giving a BT reaction in less than 5 days would mean that there was a potential for corrosion within aerobic slimes that have formed. If the SRB gives a BB reaction in less than 4 days, then there is an enhanced likelihood that electrolytic corrosion may be occurring with an increased potential for perforation of the pipes. If the IRB-BART gives a clouding-BG (CL-BG) reaction, then there is a strong potential for iron-rich slimes to have formed, while a CL-brown clouding (CL-BC) reaction with a BR occurring as well would indicate that there would be a risk of iron-rich dense plugging with a significant loss in flow. If the HAB-BART gave a DO reaction, this might mean that the well was reductive and there would be a greater risk that acid-producing bacteria would be active causing corrosion of the pipe. See Chapters 4 and Appendix B for more interpretation of the reactions using the BART testers (Table 3.3).

The information obtained from a time-based sequence of sampling for the generation of a ZIP gives a set of information about the biofouling of the well, which can be archived for later use. Zones of interrogation projection can be used forensically to determine the effectiveness of the rehabilitation to impact on the observed biofouling. Here the ZIP data and interpretations including the charts can be examined to determine whether the focus of the biomass around the well had intensified, relocated, or diminished. As a well ages, the potential for significant biofouling increases with significant potential effects on the water quality and quantity being generated in the well. It should further be recognized that this form of sampling can also be used to determine physical and chemical changes that may be occurring at the same time. This would then give an indication of the amount of bioaccumulation that has occurred or cations such as iron, copper, zinc, and manganese under more oxidative conditions and at the redox front and of chromium, arsenic, aluminum, cobalt, and cadmium accumulating on the more reductive side of the redox front. Some of these elements (such as arsenic) can continue to accumulate in the biomass around the well for years and only be released when the biomass becomes saturated with such bioaccumulates. Here the biomass enters a more extensive collapsing phase that includes the releases of these elements that had been "locked up" for a significant time within the biomass (natural filters) around the well. The sudden occurrences of arsenic (as an example) in water wells that are greater than 5 years old could be an indication that the biomass has entered a prolonged collapsing phase and the arsenic is now being released with the sloughing biocolloids (slimes) that are forming from the unstable biomass.

3.6 ROUTINE SAMPLING

Like any other system that has to be monitored as an essential part of a quality management practice, water wells should also be routinely monitored. There are

TABLE 3.3
Selection of BART Testers to Monitor Biofouling in Wells

BART	Form of Fouling	Comments	Critical TL
Sulfate-reducing bacteria (SRB)	Corrosion, black slimes rotten egg odor	Black top reaction means that the SRB are entrenched with aerobic slime formation and vulnerable. Black bottom means that the SRB are entrenched within the porous media and may be very difficult to control	<2[a] 2–4[b] 5–8[c] 9–15[d]
Iron-related bacteria (IRB)	Iron-rich plugging, encrustations, staining	Clouding (CL) indicates primarily aerobic while foam reaction (FO) means anaerobic; brown ring, brown clouding, or brown gel all indicates iron accumulation in biomass probable; red clouding indicates enteric bacteria and green clouding indicates pseudomonad bacteria, black liquid (BL) indicates a potential health risk; white base (not used to calculate TL) means carbonates are present	<2[a] 2–4[b] 5–8[c] 9–10[d]
Slime formers (SLYM)	Copious white or grey slimes	CL indicates slimes are being produced; slime rings mean aerobic slimes and dense slimes mean anaerobic slimes; cloudy plate (CP) or thread-like (TH) indicates that slime structures may be perched in the water column; and BL indicates potential health risk	<1[a] 1–3[b] 4–6[c] 7–8[d]
Heterotrophic (HAB)	Dominant when well is being challenged with organic pollutants	Up reaction (UP) indicates that the dominant bacteria degrading the organics are aerobic, and down reaction (DO) indicates that the bacteria degrading the organics are anaerobic. This would mean a greater potential for the drop in pH into acid range and the generation of gases	<2[a] 2–3[b] 4–6[c] 7–8[d]
Denitrifying bacteria (DN)	Well impacted by high nitrogen content organics with nitrates present	FO is the only reaction which signifies that the nitrate has been reduced to dinitrogen gas	<3[a] 3–4[b] >4[c]

TL, time lapse given in days at room temperature.

[a] Extremely active.
[b] Very active.
[c] Moderately active.
[d] Background activity.

already methods in place to monitor the quantity of water from wells along with some basic chemistry that might extend to pH, turbidity, and conductivity. Oxidation–reduction potential should also become much more of a routine measure since this gives a good sense of the state of the environment in and around the borehole. To extend the routine monitoring to the microbiological factors, the most common approach is to monitor for the presence of coliform bacteria. Regulations may define the level of tolerance from a zero tolerance of all total coliform bacteria to a much narrower requirement that there are no fecal coliform bacteria present to a narrower scope that restricts the test to either the thermotolerant coliforms or *Escherichia coli* itself. It is commonly thought that if there are no coliform bacteria present, then there is no biological problem with that well. This is not true since there are many other microorganisms that can infest the water well and cause production problems without the presence of any coliform bacteria. Coliform bacteria can be viewed as of great regulatory significance, since they have for the last 100 years, provided an excellent early warning of the potential health risks to the consumers of that water. These bacteria do not provide any significant information on the biofouling of the well beyond this regulated health risk.

If water wells are to be monitored for the risk of biofouling, then this would involve a completely different sampling sequence to that demanded by regulators for the coliform bacteria. Routine sampling, while including the testing for coliform bacteria, also has to include testing for recognized biofouling events that could occur in that well. The three factors at play here are as follows:

1. Need to select suitable indicator microbes that would signal a specific type of biofouling event.
2. Need to define what microbes would be specifically important in the assessing of when biofouling becomes significant and the data that would trigger concern.
3. Sampling has to be in a routine manner that is responsive to a risk when it is recognized as being potentially significant.

There are a wide number of potential candidates for indicating that a biofouling event is occurring within the water well. Selection has to be related to the perceived risk from the chemical and biological data that have been collected. Routine sampling should utilize the most suitable indicator groups for the possible biofouling that might occur. The sequence with which the routine testing can be performed is more a reflection of the severity of the biofouling that has been determined by the tests.

Generally a routine test should be conducted every 2 months, but if there is no evidence of significant risks, it can be scaled back to every 6 months or a year. If problems are identified, then the response should be firstly to do duplicate tests to determine whether the problem is getting worse [e.g., the time lapses (TLs) are getting shorter], and if the duplicates both indicate that the problems are getting worse, then a preventative maintenance servicing treatment can be applied and a fresh sampling and testing completed after the treatment. The posttreatment sampling should be left until at least 3–6 weeks after the treatment. This method

was first used in Waverley, Tennessee, from 1989 primarily using IRB- and SRB-BART testers.

Assessment of when the biofouling of a well becomes significant can be done in two ways. The first symptom that the well is becoming biofouled is the fact that the TL begins to shorten. Now it is given that there is always a potential for some biocolloidal particles to float into the water being sampled and give a false short TL indicating a greater level of activity. If the TL is seen to shorten, then duplicate samples should be taken. If both of these samples also show the shorter TL, then that confirms there is indeed an increase in the biofouling in the well. If the duplicates show the longer TL, then it means the first sample had given a false-positive response. In the Waverley scenario, SRB-BART tests would have a TL of 12–15 days indicating that a background level of SRB activity was occurring. Every 2 months, these tests were repeated, and if the SRB-BART dropped to less than 12 days (TL), then duplicate tests were performed. If these confirmed the shorter TL (e.g., 8 days), then a preventative servicing was undertaken. When the well was tested again, success would be confirmed where the TL now returned to greater than 12 days. This happened repeatedly over a 15-year timespan except on one occasion when the SRB-BART TL continued to drop indicating that the biofouling was again taking over the well and affecting water quality and quantity significantly. On this occasion, the well was rehabilitated using BCHT™ and the SRB-BART tests then returned to >12 days to signal a successful regeneration of the well.

Every water well is unique and generates its own environment that is different to the other wells around it. While each well would have its own sphere of influence defined as being that body of water, which is directly influenced when the well is pumping, the well would also be affected by the spheres of influence from neighboring wells. When the well is actively extracting water from the aquifer, a cone of depression will form if the rate of extraction exceeds the rate of replenishment of groundwater from the surrounding aquifer. If other wells in the neighborhood are also extracting water, then the spheres of influence for each of these wells may overlap and with this could come changes in the biological characteristics of the well under evaluation.

4 Symptoms of Failure, Early Warnings, and Eventual Catastrophes

4.1 INTRODUCTION

Water wells, of all "living" creatures, do not show clear signals of impending failure. As you read the word "living creatures," it is natural to think of the well as being just a borehole in the ground extracting water from an aquifer in a physical manner (by pumping) and experiencing the chemistry that would be associated with the groundwater moving from a reductive to a more oxidative regime. Two issues here would be

1. Effects of the oxidation of the water (ferric iron being a more obvious example) that could be biologically controlled and
2. Movements of solids toward the well which then become locked within the porous medium directly impacting the water flow toward the well controlled by the biomass (plugging). If there is a physical blocking of flow then this is referred to as clogging.

Until the 1980s, it was generally thought that the oxidation processes were purely chemical and indeed many, including some regulators, considered the water coming through to a water well was essentially sterile and so could not be considered living in any sense (unless of course the groundwater came under the direct influence of surface waters and became contaminated with some of the more nasty surface dwelling microbes such as the coliform bacteria).

Now, we are in the twenty-first century it is becoming very evident that not only are the wells subject to the activities of microorganisms but even the aquifers and the underpinning geological formations also contain active microbial activities that operate on a scale generally much less intense than that found on the surface. Water wells form conduits between these two worlds (subsurface aquifers and surface biosphere) that offer a much more favorable environment for growth than the aquifer itself. This is because the well is bringing a more oxidative environment down into the more reductive aquifer environment. One factor that is very obvious when working with water wells is that the interface between reductive and oxidative conditions (sometimes called the redox front) is a "magnet" to many microbes since

they can get oxygen on one side (oxidative) and nutrients from the groundwater passing out of the reductive side of the front. Changes also occur in the redox front (transitional zone between oxidative and reductive groundwater states). Unfortunately, the site for all of this activity went virtually unrecognized for years and the concept that water wells were alive with microbial activity was not taken seriously.

"Out of sight, out of mind" summarizes, in general, the industries attitude to the biofouling of water wells. If the water well is to be perceived as a living "creature" then clearly you need to maintain the health of that "creature" so that it can continue to perform for years to come (i.e., become sustainable). Traditionally, the best manner to determine the health of the "creature" down the borehole is to inspect it looking for physical signs of failure (that might not be related necessarily to the microbes down the well) and for evidence of growth that could signal plugging, water quality, and corrosion problems. The best manner to look downhole is to use the video borehole log technique to examine the changes that have possibly occurred in the well since it was last inspected. It has to be remembered that most of the growths occurring down that borehole are still not visible even with a camera that can look sideways!

In the determination of the risks of biofouling, there are clearly a number of issues that need to be considered with respect to the recognition that a problem is developing. In this chapter, different aspects of the challenge will be addressed beginning with the value of considering sustainability as being a key note issue in the management of the well or well field. This is followed by the need to examine the history of the well (archival records) and the visible state of the well (video borehole logging preferably using a high resolution color camera with an articulated lens). Finally, there is a focus on the factors that can affect the functioning of the well that could lead to failure.

4.2 SUSTAINABLE WELLS

Sustainability is another term that recently has become established in the field of well maintenance and management. The concept requires accepting that the well is inevitably going to become subject to biofouling and geochemical challenges that to some extent can be predicted and treated successfully. The application of preventative maintenance servicing should be the prime method to control these conditions, extend the life of the well, and therefore make it sustainable. If the well is monitored (to determine the state of geochemical clogging and biological fouling) and treated periodically as required to control the occurrence of these events, then the life span of the well can be significantly extended.

Examining the available historical performance data for a well is the first step in the diagnosis of the likely cause(s) of performance losses. There is a common tendency for many wells to exhibit erratic forms of decline in performance. This variability, particularly during periods of erratic and sharp declines, can be used to determine whether the failure is biological plugging, geochemical clogging, or some combination of these two events. Biological plugging usually causes more variations associated with the nature of the biomass growth cycle. In general, the

bacterial activity associated with biological plugging can become significant before there are losses in performance. This would be due to increases in aggressivity (activity) of the particular bacterial consortia infesting the well site. Where geochemical clogging dominates, the bacterial activity would not precede the decrease in production but follow later. Variability in the slope of the decline is also likely to be less where a geochemical clogging is dominating the losses in production. It should be remembered that the "As constructed" well construction records can be used in well maintenance to provide a basis for the comparison of past and present conditions and for use in other relevant calculations. As a minimum, diagrams should contain the following information:

1. An accurate geographic location.
2. The precise designation used by the project.
3. Accurate depth, diameter (including different components), casing and screen material type, screen slot size, and screen length for the well.
4. Filter pack type, particle size, and dimension.
5. Grout type and dimensions.
6. Bentonite seal type and dimensions.
7. The descriptions of the well equipment and dates drilled and developed.
8. Pump placement.

With respect to the well boring logs, these should include precise geographic location and boring identification (with cross-reference to subsequent well designations), accurate formation descriptions (including sediment and rock descriptions provided according to uniform accepted standards with accurate depths), and particle size descriptions of water-producing/accepting zones. There should also be a lithologic log (this is a record of the character, depths, and thickness of geologic materials encountered by the drill as the borehole is advanced, with emphasis given to hydraulic properties of the materials). This lithologic/boring log should contain as a minimum the following:

1. Depth at which recognizable geologic changes occur should be logged along with the depths from which samples are collected and described.
2. Description of cutting samples collected at every change of geologic materials and at 1- to 10-m (3.28- to 33-ft) intervals, and 100% logging for the screened interval in either the pilot or the final boring.
3. Changes in drilling action, that is, penetration rate, fluid loss, drilling noise, etc.

There also needs to be descriptions of unconsolidated sediments and it should note dominant grain size, sorting, and estimate of the relative percentage of sizes according to the Unified Soil Classification System procedures and those described in ASTM 421 and 422. Grain shape and rounding are useful for estimating hydraulic properties. Color can relate to the degree of weathering and oxidation–reduction potential is useful in determining degree of saturation. The depth at

which saturated conditions occur should be noted along with descriptions of consolidated bedrock with particular note of the degree of cementation, induration, and fracturing. Changes in drilling fluid properties (gains or losses of fluids, changing specific gravity, etc.) should be noted, as they provide information on water-bearing zones.

The first step should be to conduct a complete visual inspection including camera logging of the borehole, screens, and any formation material that may be visible. Often this visual inspection gives an indication of the state of the water column in the well, the degree of biofouling present, and the form and nature of any encrustations and crystalline deposits. This inspection may reveal design or mechanical failures, i.e., intrusions of fines into the well, broken or damaged screen, etc. A camera log that gives images identical to the initial well development log (i.e., pristine and new looking) does not mean that changes have not occurred. It may mean that any biological and/or chemical activity causing problems cannot be observed from the borehole itself, because these events are happening back in the pack or further out into the formation. Careful comparisons have to be made with the past and present video borehole logs while noting any changes.

4.3 HISTORICAL RECORDS

Historical records can reveal significant shifts in specific parameters that can be used to give an indication of fouling. Functioning wells are likely to be biologically compromised to some extent during their operational life. Some of these impacts may even result in a positive event. An example of this is that biofouling, as it forms and matures, may often act like a biological (natural) filter and remove chemicals from the groundwater that have potential value to the microbial consortia growing in the various biofilms. During this phase, the chemistry of the groundwater may shift significantly for the better to reflect the activity of these microorganisms. For example, groundwater with an iron concentration of 1.4 ppm may enter an extraction well and lose most of the iron through bioaccumulation. If an order of magnitude reduction occurs then the groundwater pumped from the well may contain only 0.14 ppm total iron (a 90% removal). However, once the biofilms mature then the total iron concentration held as accumulates could range from 20 to 36% by dried weight of the encrustations. As the iron levels rise, there is a reduced stability in the biofilms and some of the iron shears away with the collapsing biomass. This iron reenters the water in a biocolloidal form to elevate the total iron level in the effluent from the well. It is therefore quite common to see the total iron concentrations in the product suddenly raise in an erratic manner to as much as 10 times the original total iron concentration (e.g., 14.0 ppm). Low iron values in a groundwater sample do not mean necessarily that the water has a low iron content but simply that any iron that was in the groundwater could have been significantly removed through bioaccumulation into the biofilms forming the plugging.

Direct evidence for the role of microbes in the losses in the specific capacity (Q/s) for these wells can also be obtained by periodic direct sampling of the water in

the injection and/or extraction wells. Care should be taken to assure that the sampling method is consistent and appropriate to the particular well from which the data was/is being collected. Changes in the sampling procedure can seriously affect the data, compromising the ability for historical comparison of data. In historically reviewing the risks of biofouling using the BART testing technique, there are two key components for each bacterial group being evaluated:

1. Shifts in the time lapse.
2. Changes in the reaction patterns being reported.

Time lapses are normally measured in days through daily observation of the tests. It should be noted that the manufacturer is now providing:

1. Video camera-based reader system (V-BART-READ) whereby the operator of the tests is able to view the status of the BART tests at any time and record time lapses and reactions as they occur.
2. Microprocessor controlled testing apparatus (e.g., HAB-BART system) that allows automatic reading of the testers with an LCD display showing the time lapse and the reaction patterns these are based on.

Generally where the time lapse shortens significantly (by 1 day or more) then the bacterially induced fouling in the well is increasing. These systems provide a useful in-field, in-office, or in-laboratory technique to determine the nature of the bacteria that may be linked to the biofouling of the injection and/or extraction wells (Table 4.1).

Reaction pattern signatures (RPS) define characteristics of the bacteria that are aggressive in the sample taken from the site. The RPS can be automatically deciphered using the BART-SOFT software following the direct observation of the testers or the use of the V-BART-READ system. For the HAB-BART system, data can only be generated on the heterotrophic bacteria. BART-SOFT (the software program interprets time lapses to populations for 22 ± 1°C temperatures) are both available through the web site www.dbi.ca. This enables the data to be conveniently filed and used to generate an archival record of the state of the wells after rehabilitation. In the evaluation of a well, a number of events associated with the maturation of biofouling can occur.

4.4 BIOLOGICAL ASSAY DATA

Of all of the data generated in connection with water wells, it is the biological data that is perhaps the most difficult to obtain in a meaningful manner. An extraction well essentially draws groundwater to the borehole, and so the biological activity tends to be driven by the microorganisms moving in with the flow. In injection wells, water is pumped into the well thus displacing the groundwater and introducing the microorganisms present in the injected water into the well environment. In an injection well, this would mean that any microbial growth and biofouling might be

TABLE 4.1
Expected Concentrations in Groundwater of Various Chemicals of Concern During the Biofouling of Extraction and Injection Wells

Chemical[a]	Maturation State of a Biofouled Well		
	Young	Maturing	Biofouled
Iron	Low	Variable	High
Manganese	Low	Low–variable	Variable–high
Total organics	Low	Low	Variable
Hydrocarbon fuels	High	High	Medium–low
Recalcitrant organics	Low	Low	Low–variable
Total nitrogen	Low	Low	Low–variable
Total phosphorus	Very low	Low	Low–variable
Sulfate	NA	NA	NA–low
Cationic radionuclides	Low	Medium	High
Anionic radionuclides	Low	Low	Medium
Toxic organics	Low	Medium–variable	High–variable
Hazardous wastes	Low	Variable	Variable
Chloride	NA	NA	NA
Calcium	NA	NA	NA–low
Magnesium	NA	NA	NA

[a] Chemical references to only those wells in which the source groundwater contains those specified materials and not to all wells.

Abbreviation: NA, not affected by that state of biofouling; Low, the concentrations are reduced more than 20% as a result of accumulation within the biofouling; Variable, there is instability in the concentrations in the production water because of variability in accumulation and releases (due to sloughing) of the chemical(s) in the biofilms; High, means that higher chemical concentrations ($>20\%$ above background recharge water levels) are emerging in the production water in the sloughing materials from the biofouling that is currently entering into the zones of biofouling around the well (recharge water).

more affected by surface microbial contamination/infestations during injection well operations. Beyond this major difference in the likely source of microbial biofouling, the environment in and around the borehole and the manner in which the wells are operated are more likely to influence the types of microbial problems that may develop.

To conduct a biological assay, there is a need to get a general evaluation of the levels of bacterial aggressivity in the groundwater and, in the case of injection wells, of the water being injected into the ground. The simplest manner to get a first level evaluation of this aggressivity is to use the biological activity reaction tests (BART testers, DBI, Regina, Canada) in which time lapse (to the observation of the first recognizable reaction) forms the critical criterion. Time lapses are measured as the time delay from setting up the test to the first appearance of a visible reaction generated by the bacteria. It is possible to use this time lapse to determine how aggressive the bacteria are on the basis that the shorter the time lapse the more aggressive the populations of detected bacteria are. Conversely, the longer the time lapse then the

less aggressive the bacteria are. The units commonly used are days but under very active conditions then the time lapse can be measured in hours or even seconds.

There is a wide range of bacteria that can become aggressive in the wells. The full range of bacterial groups (consortia) includes the following by group name, acronym, and the culture medium used to detect the bacteria (Table 4.2):

Iron-related bacteria (IRB) applying Winogradski medium.
Sulfate-reducing bacteria (SRB) applying Postgate's medium.
Slime-forming bacteria (SLYM) applying glucose peptone medium.
Heterotrophic aerobic bacteria (HAB) applying sugar peptone medium.
Algae (ALGE) applying Bold's medium.
Fluorescent pseudomonads (FLOR) applying peptone base medium.
Denitrifying bacteria (DN) applying nitrate peptone medium.
Nitrifying bacteria (N) applying ammonium salts medium.

Note: The term "applying" means that the selected culture of these bacteria is based upon the medium described that is in common use in microbiology. Of these bacteria, the groups most commonly investigated are:

IRB (associated with iron concretions plugging up the wells).
SRB (associated with anaerobic conditions with the production of hydrogen sulfides and iron sulfides with black waters and slimes produced).
SLYM (sensitive to the broadest range of bacteria that can occur in wells).

Where a well is being compromised by organic loadings under aerobic conditions, there are likely to be aggressive loadings of heterotrophic bacteria and the HAB test should be used. If the injection well is recovering water from an aerobic biodegradation facility, there is likely to be an aggressive pseudomonad population and the FLOR test could be used if it is necessary to identify the pseudomonad bacteria in more detail. Many of these bacteria often dominate the aerobic degradation of a wide range of organics including hydrocarbons (such as JP4, diesel, gasoline) and solvents. At the same time, pseudomonads can also cause biofouling in the wells. The activity of these bacteria can be measured by their

TABLE 4.2
Likely Aggressivity/Activity of the Five Most Common Consortia of Bacteria (Left-Hand Column) at the Four Stages of Maturation in a Plugging Well (Going from Young to Plugged, i.e., Column 2 to Column 5)

Consortium	2, Young	3, Maturing	4, Biofouled	5, Plugged
Iron-related bacteria	Low	High	High	Medium
Sulfate-reducing bacteria	Very low	Low	Medium	High
Heterotrophic aerobic bacteria	High	High	Medium	Low
Slime-forming bacteria	Medium	High	Medium	Medium
Denitrifying bacteria	Low	Medium	Medium	Low

aggressivity when samples are tested as being high, medium, or low depending upon the time lapse observed during testing.

As a well undergoes biofouling toward a total plugging, the probability of a successful regeneration decreases and the consortia of bacteria causing the plugging frequently changes. This can mean that a treatment effective against one consortial group of bacteria may be less effective against another group. There is also likely to be changes in the aggressivity of the bacteria in water samples likely to be collected immediately downstream of the site of biofouling. The designation does not relate to the aggressivity (activity) of the bacteria inherent within the biofouling biofilms but only the probability of detecting the bacteria in the discharge water:

1. Low, meaning that the bacterial activity is less likely to be detected at this stage even though the bacteria may be very active in the biofouling.
2. Medium, meaning that there is a reasonable probability that the bacterial consortia will be in the produced water if present and active in the biofouled well.
3. High, meaning that there is a high probability that if the bacteria are active at the biofouled sites, they will be detected with a high level of activity.

Much of the biofouling is caused by the formation of biofilms. These growths commonly undergo cyclic stages of biofilm growth followed by sloughing and compaction. Flow volume changes can be impacted by these harmonic shifts during the maturation of the biofilms. In laboratory studies, it has generally been found that the cyclic form of biofilm growth does affect the product flow volumes out of, or into the well. Where the loss in volume flow is linear in nature then there is a greater probability that the losses are a result of a geochemical event or some shift in the availability of groundwater to, or from, the well.

This bioaccumulation of particularly metallic cations into the biomass means that this biomass becomes a little like a "slime chromatograph" in which different cations are being accumulated at different places along the biomass as the conditions move from reductive to oxidative.

4.5 BIOFOULING: THE IMPACT OF BIOFILMS ON SPECIFIC CAPACITY

Biofilm is the name given to bacterial growths occurring over surfaces, commonly as a continuous coating. This coating often begins slime like but often matures into more complex structures that include amorphous or crystalline encrustations, nodules, tubercles or exotic growths extending out into the water. As the biomass matures, it occupies void spaces within porous media that can drastically change the hydraulic characteristics of the water flow. Most of the biofilms in the biomass are comprised of bound water that is held in place by strands of polymers produced by the resident microorganisms. Preventative

maintenance servicing or regeneration of a well has to involve some method for disrupting and dispersing these polymers before the microbial cells can be exposed and attacked.

Application of chemicals during regeneration can be effective where the resultant reactions are able to penetrate down the water conduits deeper into the biofilms. The chemicals attack the clusters of microbial cells that commonly involve several species. Treatment chemicals entering primarily through these water conduits toward the well may also become neutralized, if they become entrapped within the polymeric webs and accumulate there.

In preventative maintenance servicing and regeneration, the state of maturation of a biofilm is critical to the form of treatment to be applied. Young biofilms begin with very high water contents, occupy a relatively large volume and are inherently unstable. Maturing biofilms have gradually reducing water content and increasing content of accumulates. This can include metallic ions with iron oxides and hydroxides often dominating under oxidative conditions. Carbonates (such as calcite) may also accumulate generally as crystalline structures synthesized by the microbes within the biofilm. Under reductive conditions, it is various metallic sulfides that accumulate, particularly iron sulfide. Organic materials can also accumulate but are also likely to be degraded (if not recalcitrant). If they are recalcitrant, it means that the conditions present do not support the degradation of the organics and so they accumulate. In a matured biofilm, the bioaccumulation in the biofilm will reach a saturated state with bound water and organics reduced to lower levels. At this stage, the biofilm becomes an encrustation and regeneration must focus on the destruction of the elements dominating that relatively dormant mass. Iron content under some oxidative conditions can be as high as 30%–36% of the dry weight mass while the organic content can fall to less than 1.0%.

Once the biofouling begins to take effect one of the very first signals that the well is in trouble is a shortening in the time lapses when the water is tested using the BART methods. This shortening in time lapse signals an increase in the levels of bacterial activity in the water sample that have been drawn from the well. Remember that the water sample will be less likely to include those bacteria that are tightly bound up in the encrustations. Microbial activity within these growths is likely to precede increases prior to physical changes in flow in the water itself.

Time lapse shortening (an increase in activity) is likely to signal the activity moving from background or low to moderate or even high. This activity burst will be reflected in a falling specific capacity (Q/s) within a period of weeks or months after the burst in microbial activity indicating that the biofouling is getting significant. It is also possible to determine the losses in water well productivity by examining changes in the drawdown. Successful regeneration means inevitably destruction of a large percentage of the biofouling biomass has occurred. This destruction means that the dispersed biomass now being pumped out of the well could contain very high levels of these metallic cations. Such discharges may have to be handled as hazardous waste should the concentrations fall into the regulated discharge range.

4.6 SIGNIFICANCE OF DRAWDOWN CHANGES

A vertical well has a head within the water column that will be stable and reflect the neighboring water table when the well is at rest. Pumping extraction wells can cause excessive drawdown of the water level when the volume of water entering the well cannot keep up with the pumping demand at the static head pressures of the well. Pumping fluids into injection wells have a reverse effect on the static head. The water level rises in the column as a result of the greater volume of fluids entering the well compared to the amount that can leave the well through the slots and/or move directly into the porous media of the aquifer. Horizontal wells function differently because all of the screen slots cover a range of depth equivalent just to the width of the screens. Hydraulic efficiency in this case would be based on a narrow range of the aquifer depth with the biofouling likely to extend along the entire length of the screen. Evidence of this occurring may be seen through the presence of gas blankets along the upper quadrant of the wells where gases are collecting but are unable to escape through the (plugged) slots or are waiting to be degraded. These events can best be examined by video logging the well.

Changes in the elevation of water levels in piezometers sited within the normal zone of influence during active pumping of the well can reflect the losses in permeability in the surrounding media that have resulted from geochemical and/or biological activities. In vertical extraction wells, this would mean that the drawdown would become greater until the pumping action was impaired by the water level reaching the pump itself. For horizontal extraction wells, there would be a loss in ability to extract water under constant pumping conditions. The reverse is true for injection wells. In vertical injection wells, the same losses in permeability would cause the water to rise in the borehole. If the well were capped then the impact would be seen in greater pressures needing to be applied in order to inject the specified volume of fluids into the well. Horizontal injection wells would show similar increases in back-pressure resistance that would lower the efficiency with which water could be injected through the well.

Operation of polluted wells is more difficult when there is a lateral plume floating on the groundwater at a depth occupied by the well screens. For a vertical well, this would mean that plume might be focusing biological activity over the depths where the plume is having a significant influence. Drawdown data, under these circumstances, may become difficult to obtain. Drawdown measurements may be further exacerbated by biological degradation and smearing of these contaminants at the interface. For horizontal wells, a condition could be reached where considerable lengths of the screens may be impacted. This would theoretically increase the ability to remove the contents of the plume but also increase the potential for severe plugging along large lengths of the well.

4.7 OIL-LUBRICATED PUMPS: THE RISKS

Another factor that can affect the early appearance of biofouling in the well is oil. Oil-lubricated pumps are a problematic source of fouling since, while the oil does a good job lubricating the pumps, it can also be a food for the bacteria. Many of the

oils can be degraded by the microbes oxidatively unless there are very powerful biocides in the oil. In which case, the biocide is probably a toxin (since it is stopping the oil from degrading). The inclusion of a biocide in the oil may be considered unacceptable in a water system that could then generate a greater health risk and/or faster rates of fouling. "Biodegradable" oils, when used for lubrication of pumps, may be even worse than the hydrocarbon-based oil since these organic vegetable oils would be broken down more quickly by the microbes as food therefore causing faster rates of plugging.

4.8 CONSIDERATIONS IN DECIDING UPON A TREATMENT STRATEGY

A failing water well needs to be treated to remove much of the biomass and get the well working properly again. Remember that the well is being fouled by a biomass growing around the well. That means you need to remove as much of this biomass. The objective of a treatment has to include destruction of the biomass to such an extent that it can be removed (by pumping as slurry) from the well leaving the well closer to its original characteristics. There are some essential stages in this treatment:

1. Provide conditions that will cause the biomass to break up.
2. Once the biomass is broken up then use physical techniques to remove the biomass slurry from the well.
3. Make sure that slurry having high hazardous accumulate content is disposed of correctly following local regulations for hazardous waste management where this becomes appropriate.

Treatment needs to involve as great a shock (chemically and physically) as necessary to destroy that cylinder of biomass (vertical or horizontal) in a manner so that it can be removed from the well.

Today this required chemistry should cause the biomass to shatter so that it can be removed. Such chemistry should not include any elements that would end up feeding the microbial survivors (and there are survivors). Elements of chemistry that have been found to be effective in aiding in the destruction of the biomass include either: (1) a radical shift in the pH down to an acid regime and/or (2) an alkaline regime as an alternative depending on the natural chemistry of the groundwater. For this function, strong inorganic or organic acids can be judiciously used (for the acid adjustment) or inorganic hydroxides (for the alkaline adjustments).

There is seldom enough chemistry to do the job. The target biomass most likely resembles a tightly woven slime ball that has to be destroyed. This can be done by destroying the polymers that form the woven fabric in the "ball." Good penetrants appropriately applied are capable of doing this. Under no circumstances should a chemical be used that has the potential to "feed" the biomass during, or after, the treatment. For example, chemicals containing phosphorus are a high risk chemical group (e.g., phosphoric acid and linear polyphosphates). While these chemicals are very good at breaking up the biomass, this becomes very problematic since the

surviving biomass can then take up and stored the phosphorus inside the cells as polyphosphates, ready for future growth.

It is interesting to note that phosphorus-based detergents were banned in the 1970s because so much phosphorus was getting into the surface waters and stimulating massive algal growths (blooms). After the banning of these P-based detergents, there has been a steady improvement in surface water quality with less summer blooming in the waters. Unfortunately, it is not possible to see the growth around the water well but just experience the effects of the fouling and so there has not been a ban on phosphorus applications applied to treat to groundwaters. At this time, significant levels of phosphorus are still being applied to wells, getting the biomass around the well and causing faster rates of growth and more frequent treatments to keep the well going! We need to be able to understand what blooms around a water well are in a manner that we have come to understand blooms in surface waters.

If phosphorus is used in the treatment process then it might be very difficult to recover that phosphorus again after the treatment. Getting out the same amount of phosphorus as was put in does not show success since the treatment would have impacted on the biomass. Recovery should be a combination of unspent applied phosphorus (if there was any) and the phosphorus that had already been bound up in the biomass that was destroyed and removed. Effective treatment would involve recovering more phosphorus than that applied based on the formula that recovery of all of the applied phosphorus and all of the phosphorus that was in the biomass around the well should be considered necessary to declare success. Applied phosphorus (as a part of a treatment) can interact with the intrinsic polyphosphates within the biomass. Here the applied phosphorus could get taken up into the biomass unless the biomass is effectively destroyed by the treatment leading to a recovery of all of the applied phosphorus and, in addition, much of the polyphosphate from within the dispersed biomass is recovered after dispersion.

Given the tortuous nature of fractures and the voids around the well, it would be most likely that there would be an incomplete recovery. The golden rules during well treatment has to be (1) "do not feed the biomass," (2) "remove the biomass," and (3) "recover all of the chemicals that had been accumulated for whatever reason in the biomass." A chemist, when conducting experiments in the laboratory, often applies heat to speed up the reactions (they do not want to sit there all day on an uncomfortable lab stool!). Heat can be a valuable aid in the treatment and regeneration of wells.

> Organisms must not be regarded as chemical reactions which take a stereotyped course under all conditions; they adapt themselves to suit the different environments in which they grow, and the adaptations are comparatively great in the case of such plastic organisms as the bacteria. One must therefore enter a caveat against the attitude adopted by Molisch who finds himself unable to believe in the existence of reproductive structures which are not forthcoming in his own artificial cultures. (David Ellis, 1919, *Iron Bacteria*, Methuen & Co. Ltd., London, p. 109.)

5 Remedies from a Drop to a Drum

5.1 INTRODUCTION

Water wells are long-term slow-reaction systems that, if not carefully monitored, can suddenly fail catastrophically. In today's world, it is common practice to believe that there is some "magic" chemical out there to "fix" any problem forever with the least amount of effort. We would all like to be able to drop a smidgeon of the magic elixir down a well and watch the water return in clarity with the flows coming back and no biomass activity down the hole. To do that, it would not be a drop that would be required but perhaps several drums of not just a single chemical but a combination of chemicals tailored to attack the "enemy" effectively. The enemy in this case is the bloated biomass that is now degrading the quality of the water and the production potential causing that well to decline. Which comes first, the loss in quality or the loss in flow? That is one of those "chicken and egg" questions. Sometimes it is the flow that slows down first and on other occasions it is the quality of the water being pumped from the well that deteriorates first. If the well begins to lose production first then that means that biomass associated with the plugging is deeply entrenched and is, perhaps, some distance from the well itself. If the quality of the water begins to decline, particularly with increases in iron content, then that means the biomass is closer to the borehole and unstable. In deciding on a treatment strategy, the position of the biomass around the well can be a critical factor in determining the approach to treating the well. There is however, two major challenges that should be addressed before the water wells are even fully developed:

1. When and how often should preventative maintenance (PM) servicing be applied to the well.
2. When has the well degenerated to such an extent that it requires regeneration.

To determine the most suitable treatment it is important to understand what is ailing the patient (in this case, the well) (Table 5.1 and Table 5.2). There are symptoms that the well will show when failure is occurring and these symptoms need to be monitored. Three primary symptoms are found in the water quantity that is being produced and the water quality that is being delivered at the well head. For the water quantity, the prime indicator is the

specific capacity (Q/s). For the water quality, the prime indicators are more numerous and include:

1. Turbidity [cloudiness or total suspended solids (TSS)].
2. Increases in chemicals in the water (particularly organics and cations such as iron).
3. Increases in the biological activity determined using biological activity reaction tests (BART) testers.

TABLE 5.1
Criteria for Determining the Need to Conduct a Preventative Maintenance Servicing

Criterion	Min. (%)	Mean (%)	Max. (%)	Comment
Q/s	0	−5	−15	Any drop in Q/s is significant and PM should be undertaken
Total suspended solids (TSS)	+5	+20	+40	Repeat testing may be necessary to confirm the TSS increase
Turbidity	+5	+20	+40	Most likely due to greater microbial activity
Time lapse (TL) sulfate reducing bacteria (SRB)	−0	−20	−40	Increases in SRB activity commonly follows those occurring from other bacteria unless a biological activity reaction tests (BART) tester (black top, BT) reaction is observed
TL iron related bacteria (IRB)	−0	−30	−40	IRB are mostly significant in wells with high iron content and these activities will precede increases in the ferric iron
TL heterotrophic aerobic bacteria (HAB)	−10	−20	−30	If the water has a significant organic content then the HAB will precede the shorter TL for the other BARTs
TL slime forming bacteria (SLYM)	−0	−10	−20	TL for the SLYM-BART testers tend to be short and, while this test is very sensitive, it may not show significant changes in the TL compared to the others
Ferric, ppm	0	+30	+60	Ferric iron can suddenly increase as the bioaccumulation of iron slows down in the biomass indicating that plugging and water quality is likely to change dramatically

Note: The minimum (min.), mean and maximum (max.) changes are given as percentile shifts upwards for the TSS, ferric iron and turbidity but downwards for all of the other criteria. All percentile changes are from the original data collected after the well has been fully developed and these characteristics were relatively stable in the operation of the well (±5%). Minimum changes would be the first signals that the well is beginning to fail. Of these criteria, it is the Q/s (specific capacity) that should be considered very seriously since, in a fast fouling well, this could drop very quickly into the realms where regeneration would have to be undertaken.

TABLE 5.2
Criteria for Determining the Need to Conduct a Radical Regenerative Treatment

Criterion	Min. (%)	Mean (%)	Max. (%)	Comment
Q/s	−15	−30	−60	When a loss is greater than 60% in Q/s it is highly unlikely that a greater than 40% recovery could ever be made towards the original specifications
Total suspended solids (TSS)	+30	+60	+90	Repeat testing may be necessary to confirm the TSS increase. There is likely to be considerable variation in a biofouling well
Turbidity	+20	+40	+60	Most likely due to intensifying microbial activity
Time lapse (TL) sulfate reducing bacteria (SRB)	−10	−30	−50	Increases in SRB activity commonly follow increases from other bacteria unless a biological activity reaction tests (BART) tester (black top, BT) reaction is observed
TL iron related bacteria (IRB)	−20	−40	−80	IRB are mostly significant in wells with high ferric-iron contents. If iron or manganese is not a factor, then this TL may not change so significantly
TL heterotrophic aerobic bacteria (HAB)	−30	−50	−70	If the water has a significant organic content then the HAB may be the better biomarker for severe biofouling in the wel
TL slime forming bacteria (SLYM)	−10	−20	−30	TL for the SLYM-BART testers tend to be short. Because of the sensitivity of this test, the TL may not decline so drastically when severe biofouling is occurring
Ferric, ppm	30	+60	+90	Ferric iron is most likely to be released in wells with severe biofouling since the iron concentrates closely to the oxidative side of the redox front and is easily released by the maturing iron-rich biomass

Note: The minimum criteria shown above are similar to the maximum shown in Table 5.1 and show the extent of the deterioration that is most likely to trigger the need to undertake a regeneration of the well. Again, the longer the time that is allowed to elapse before regeneration is applied then the more challenging that treatment becomes and the less likelihood is there of success.

The bottom line is that the well has to be monitored to ensure that PM servicing is performed before the well becomes too fouled that regeneration has to be undertaken. The next three sections deal with PM servicing, regeneration, and then the response to a water well that is failing but has not been subjected to any routine reactive monitoring.

5.2 PM SERVICING

Preventative maintenance is designed to be a more routine treatment that would be performed to ensure that the well continues to operate close to its original performance. Here the first rule would be that routine monitoring should also be interpreted and not just filed away and forgotten. Preventative maintenance should be applied as soon as there is evidence that the well is beginning to fail through either diminishing production or falling water quality standards. Preventative maintenance should be applied when any of the standard criteria for the well are compromised. Of these various criteria, it is the Q/s that is the most significant since this clearly indicates that the water well is losing its ability to produce water. However, this is not always the first signal. Commonly the order with which failure will occur begins with the time lapses for the various BART testers. Time lapses begin to shorten, indicating greater levels of microbial activity. It should be remembered that the occurrence of these bacteria in the pumped water most commonly follows after that bacteria have established their communities within, and around, the well in the form of slimes, encrustations, biofilms, and tubercles. This means that the occurrence of these microbes in the water is evidence that they are now fully established colonies (downhole) and we are seeing the attempts by some microbes to move to new environments. Signals that this is happening go across the full spectrum of criteria. Other increases in the TSS, turbidity, and the appearance of ferric iron in the product water. For the shortening time lapses, it is commonly the slime forming bacteria (SLYM) and the heterotrophic aerobic bacteria (HAB) testers that will show this happening first. Sulfate reducing bacteria (SRB) testers will generally be slower in the detection of biofouling in the well particularly if the SRB are giving a black base in the detection reaction which reflects the potential that these bacteria are covert, living deeper within the reductive porous zones of the formation. Time lapses for the IRB-BART may occur coincidentally or slightly before there is an increase in the ferric iron in the product water.

There are a number of criteria that may show a PM treatment is desirable with the objective being to return the well to its original production and quality criteria. Like all routine maintenance servicing activities, it is easy to put it off for another month or year since the water well still appears to be performing well. Even in very large corporations it is often very difficult to justify the expenditure to, what appears to be, just "tweaking" the well. This tweaking actually controls the biofouling before it reaches a point when much more expensive regeneration of the well or even abandonment and replacement occurs. In the era of deep pockets and disposability, such an attitude has been commonplace and PM or regeneration treatments have not been considered necessary by many. There are many abandoned wells around the world that could have had a much longer service life had PM been applied along with regeneration when necessary.

Today the interest in sustainability is growing. Sustainability may be defined as "to maintain or keep going continuously"; in other words to keep the system functioning for a long period of time. For water wells, the life span can be from as little as two years to as long as fifty or more years. Applying PM regularly and automatically makes the well more sustainable since now it would have been

maintained to operate continuously at closer to the original specifications of that well. Failure to perform PM means that the degeneration will occur without check until the wells performance becomes unacceptable whether that is at 20% loss or even as much as a 98% loss in Q/s.

5.3 REGENERATION

Regeneration is designed to be a much more rigorous treatment of the well that has suffered from a more serious failure that the regular PM was not able to correct. This should be a relatively rare event occurring only when the PM has not been able to recover the original characteristics of the well then failure is more of a probability. It would often be the first event in wells that have already become so biofouled that specific capacity has been reduced significantly. Threshold criteria can be defined that would be used to determine when this more radical treatment (regeneration) would have to be undertaken to rehabilitate the well. In comparison, regeneration has to be much more vigorous as a treatment than PM since PM would already have been applied and failed to return the well to its desired state (close to original specifications for the well when newly developed). It is inevitable that the chemical and physical treatments of the well would have to be sufficiently powerful to ensure a disruption and dispersal of the biomass that is now significantly affecting the performance of the well.

5.4 WATER WELLS THAT FAIL WITHOUT ROUTINE PM OR REGENERATION

There are many water wells out there where the operators have not considered the full impact of failing to treat the well other than to ensure that it is mechanically functional and produces water that can be handled by the treatment and distribution system in a manner that would make it useable to the end consumer. To bring one of these wells into a routine PM and regeneration program (as required) has one major challenge apart from the complacency generated by the well(s) appearing to function anyway. That challenge stems from the lack of data. It is not possible to reconstruct the original characteristics of the well unless there was a history based upon valid archived data.

It has to be recognized that in these cases of neglect, examining the available historical performance data for a well is the first step in the diagnosis of the likely cause(s) of performance losses. There is a common tendency for many wells to exhibit erratic forms of decline in performance. This variability, particularly during periods of erratic and sharp declines, can be used to determine whether the failure is biological plugging, geochemical clogging, or some combination of these two events. Biological plugging usually causes more variations associated with the nature of the biomass growth cycle. In general, the bacterial activity associated with biological plugging can become significant before there are losses in performance by the well due to an increase in aggressivity of the particular bacterial consortia infesting the well site. Where geochemical clogging dominates, the bacterial activity does not precede the

decrease in production but follows later. Variability in the slope of the decline is also less where a geochemical clogging is dominating the losses in production.

Beyond the primary indicators of the cause for production losses, there is the more detailed assessment that is inherently essential for the regeneration of the wells to assure acceptable performance. After an unacceptable loss in production, a two-phase reaction program needs to be instituted. This will cost money and effort but will give sustainability to the well. The two phases in the reactive treatment program are:

1. First phase would be a regeneration treatment to return the well to an acceptable level of performance.
2. Second phase would be to set up an ongoing PM servicing strategy that will assure that, with a minimum of treatment applications and effective monitoring, the well remains within an acceptable range of performance.

The first step to conduct a complete visual inspection includes camera logging of the borehole, screens, and any formation material that may be visible. This visual inspection only gives an indication of the state of the water column in the well, the degree of biofouling present, and the form and nature of any encrustations and crystalline deposits. This inspection may reveal design or mechanical failures, i.e., intrusions of fines into the well, broken or damaged screen, etc., A camera log that gives images identical to the initial well development log (i.e., pristine and new looking) does not mean that changes have not occurred. It could mean that any biological and/or chemical activity causing problems cannot be observed from the borehole itself because these events are happening further back in the pack or out into the formation. Careful comparisons have to be made with the past and present video logs while noting any changes.

Historical records can reveal significant shifts in specific parameters that can be used to give an indication of fouling. Functioning wells are likely to be biologically compromised to some extent during their operational life. Some of these impacts may even result in a positive event. An example of this is that biofouling, as it forms and matures, may often act like a biological filter and remove chemicals from the groundwater that have a potential value to the microbial consortia growing in the various biofilms. During this phase, the chemistry of the groundwater may shift significantly to better reflect the activity of these microorganisms.

Injection wells with a high total iron content (in excess of 0.5 mg/L) in the injection water can be expected to generate an ongoing plugging rate since the iron is moving from the borehole through the limited well screen surface outwards into the formation. This can then generate very significant localized plugging that could lead to rapid losses in hydraulic conductivity, rising injection pressures to achieve targeted flows, and a shortening of the operational life span. This problem would be exaggerated if the injected water were oxidative (containing oxygen) and was then forced out into the formation causing a redox front to develop away from the well. This could then become a focused site for biomass growth.

Historically, iron is the most well known chemical parameter that causes well clogging problems including losses in production and deterioration of water quality.

This failure in production used to be considered mainly a chemically driven event. It was thought that the oxidative conditions created a chemical precipitation of the iron into large masses of ferric oxides and hydroxides, perhaps with some carbonates. These structures commonly resulted in encrustations that were hard and brittle, yet very porous. It is important to recognize that some primary features inherent in the failures of wells are microbiological.

Formerly scientific research emphasized the chemical and physical factors more than biological in well fouling. Recent research and field experience demonstrates that the impacts on a well are primarily driven by microbiological events. In a semi-saturated environment, the microbial activity tends to be dominated by the molds (fungi). For conditions where there are plumes of organics floating in the groundwater on top of the saturated media. Fungi may play a major role in the accumulation and degradation of these floating organics. Volatile materials escaping from the water into the soil's atmosphere in the vadose zone may also be bioaccumulated and biodegraded by these fungal growths.

In the groundwater saturated zones, there is likely to be significant oxidation–reduction potential (ORP) formed between the more oxidized conditions (commonly above and around boreholes) and the reduced conditions (commonly back in the aquifer formations). This shift in the ORP from an oxidative to a reductive state is referred to as the redox front. This front is likely to be dominated by various species of bacteria growing in communities known as consortia. The exact nature of the consortia growing at a specific site will be dependent, in part, on the ORP at the site. Commonly, the dominant microorganisms shift from oxidative through the redox front to reductive in the following sequence: iron related bacteria (IRB), methane consuming bacteria (MCB), HAB, SLYM, DN, SRB, and MPB (methane producing bacteria) with the HAB, SLYM, and DN dominating at the redox front. Both DN and MPB can produce gases (nitrogen or methane, respectively) causing temporary foam blocking of the porous media. This can result in sudden (often short-lived) losses in flow. Nine forms of biological growth can be seen in camera-logging wells. These are

1. Well snow which are particles in almost a gel-like state;
2. Calcite stalks;
3. Slime-like globular structures;
4. Nodules or tubercles;
5. Encrustations;
6. Loosely attached slime which easily detaches and floats through the water;
7. Threads;
8. Ill-defined fragile clumps of slime that extend out into the water;
9. Concretious arches that can extend right across the screen's diameter.

In video camera logging of an older well then these growths could be used to determine how severely the well had become biofouled.

Since the microbiology interacts with the chemistry and the physical states of both horizontal and vertical wells, it is often difficult to obtain a comprehensive answer to these sudden partial or complete failures. It could be argued that a comprehensive database is essential. It could also be argued that for many of the existing wells such an argument makes no sense since the data was not historically gathered and the cost of data acquisition could be seriously questioned.

One group of chemicals that has been historically used in the treatment of wells is phosphorus and there is a probability that some of these wells that had not been subject to ongoing data management may have been treated with these chemicals. Phosphorus has been well recognized as a cleaning agent that dominated the detergent industry until the recognition that it was a major nutrient that supported the algal blooms in surface waters. The banning of phosphorus in detergents created a reduced occurrence of pollution associable with the use of these chemicals. However, blooms in water wells are not so easily recognized or quantifiable except through drops in the specific capacity and failing water quality commonly with increasing microbial activity. These failures can be linked to biological growths not only down the borehole but also outwards into the formations. Application of phosphorus stimulates this growth and accelerates the rate of failure in both specific capacity and water quality. Forms of phosphate that could significantly impact a water well causing exacerbation of biofouling problems is not limited to phosphate (PO_4) but also includes any organic and inorganic forms of phosphorus including polymerized forms and phosphoric acid.

Advocates for the application of phosphorus to water wells assert that, as a part of cleaning strategies, treatment effectiveness is enhanced since all of the applied phosphorus is totally recovered during the later stages in the treatment process. However, reality could be that the applied phosphorus (of any type) can readily be converted by the surviving biomass to phosphates and polyphosphates that are then accumulated. This would be particularly probable in ground waters passing through organic-rich zones such as ancient alluvial river deposits. Such materials are likely to become more biologically active during an associated treatment causing shifts in the in situ ORP that could trigger extensive biological activity and stimulate greater levels of phosphorus accumulation. In the event that a strongly acidic phosphatic material was applied such as phosphoric acid, the biomass focused within the alluvial deposits would still accumulate the phosphorus through a buffering action within the biomass. Such events as those described above would result in the waters produced, after treatment, having a zero phosphorus balance not because all of the phosphorus applied has been recovered during the treatment process but because the phosphorus has now become locked up in the biomass and would then be readily available to stimulate a down hole bloom. It is very logical that the ban on the use of phosphorus in detergents should be extended to include all well treatment agents containing any significant amount of phosphorus. Such regulatory initiatives could lengthen the expected life of water wells with a reduced potential for biofouling.

Other problems can arise when oil-lubricated pumps have been installed in water wells. While hydrocarbon-based oils have long been used as lubricants, they do leak out into the borehole and can degrade. This can lead to too numerous to count bacterial numbers that cause the regulatory rejection of the well.

Hydrocarbon oils have been replaced to some extent by vegetable oils with one of the selling features being that they are biodegradable. It is a logical extension of that environmentally friendly argument to raise the question "what happens to the microbial biomass that has degraded the vegetable oil which, as a result, is gone from the well?" The answer has to be that vegetable oil has radically stimulated the growth of microorganisms downhole and so presented a greater risk that could become a regulatory concern. For the hydrocarbon-based oils, the degradation is slower because of the complex nature of the polymers in those oils. Both types of oil will break down but the more degradable vegetable oils will tend to degrade faster and potentially stimulate biofouling activities much more quickly. These oils are used to lubricate the turbine pumps and any spillage into the borehole is likely to occur as a potentially significant biofouling event. In the regulation of these oils, it has to be recognized that both types of oil present a risk to the microbiological health of the well with the vegetable oil, in all probability, having a more immediate impact.

Where there has been water quality monitoring, there has been a reliance on simple inorganic chemistry to determine the "health" of a well. Chemical data has often been limited to a range of metallic ions including sodium, potassium, calcium, magnesium, and iron, chloride ions, sulfate ions, and a broad sweep of the total dissolved solids using conductivity. This type of analysis was slanted toward determining the origin of the groundwater and its hardness rather than determining the likelihood of biological and/or chemical challenges to the performance characteristics of the well. This means that available historical data may not be too relevant to the comparative assessment of the need for PM or regeneration of the well.

It has to be remembered that addressing biofouling/performance problems in horizontal, extraction, and injection wells is made complex by a set of factors that interact to make interpretation more experiential than scientific. In determining relative importance in the historical data, various factors will be addressed in a logical order. This would involve the experiential knowledge and understanding of the site staff as much as the need to follow a preordained pathway without consideration to local conditions that could make each well unique in the manner that they are treated. Here the message would be to listen to the operators of the well(s) for they are sensitive to the ways in which those wells perform and have knowledge that might not appear in any diagnostic book.

Performance is often designated as compromised when the hydraulic conductivity within the porous media around the well becomes severely curtailed by a growth of biofilms with, or without, associated encrustations that biologically plug off the well. Geochemical clogging can also cause this loss in conductivity and, under many circumstances it can be a combination of both biological plugging and chemical clogging. On contaminated groundwater sites, the prime concern is the control of the contaminants by the extraction and/or injection wells sometimes with relatively little or no attention paid to the basic hydraulic performance of the well.

6 Regeneration from Edge of Catastrophe

6.1 INTRODUCTION

Everything in well management has to be put into perspective. In the perfect world, the water well would treated every 6 weeks as a preventative servicing routine and would never fail. This is not a perfect world and things fail sometimes suddenly with no warning signs. Water wells are complex creations that can be affected by any combination of physical, chemical, geological, and biological factors not necessarily in that order. There are five broad categories into which well problems can be grouped.

6.1.1 Structural or Mechanical Failure

Structural or mechanical failure is due to natural stresses exceeding the designed capacity of the equipment. It exists either because of a failure to design and/or install the wells properly, or because the wells were impacted by some shifting in the soils and/or the geological strata in the region. This group of problems is most likely to be a well-specific event and not involve either clogging or plugging. Improper development, poor design of filter packs or screens, or over pumping can cause physical failure of extraction wells.

6.1.2 Clogging

Clogging is due to geochemical impacts dominated by physical and/or chemical factors. Geochemical clogging is driven by a mixture of materials created by chemical interactions that occur in the void spaces around the well and by materials such as clays, silts, and sand (fines) that clog the voids as they move towards the well with the groundwater flow. It was historically thought that clogging was the major cause of well fouling and it commonly involved an oxidative precipitation of chemicals, in particular ferric iron and carbonates, into insoluble accumulates. Changes in physical conditions in the well caused by turbulence often affects pCO_2 and/or O_2 leading to mineral precipitation. Sand, silts, and clays were thought to accumulate to cause the clogging. Now it is recognized that many events previously characterized as geochemical clogging have been recognized as biologically driven plugging events.

6.1.3 Plugging

Plugging is due to the infestation of the well and/or the surrounding environment with excessive levels of microbiological activity. Biological plugging can start with the initial growth of biofilms at sites where the growth restricts groundwater entry into the borehole. This initial growth matures and hardens through the process of bioaccumulation particularly of iron and other metallic cations, and through the synthesis of crystalline structures usually based on carbonates. At the same time this maturation occurs, there is an entrapment of clays, silts, and sands that add bulk to the volume and the mass. This entrapment then increases the losses in groundwater flow through the infested region.

6.1.4 Corrosion

Corrosion is related to microbial activity impacting screen and pump integrity. It is lumped in with plugging since, on most occasions, the corrosive processes follow the generation of a biofilm that would commonly be contributing to a biological plugging process.

6.1.5 Regeneration

Regenerationas a term refers to "restore to effectiveness" or "restore to a functional condition." For the regeneration of water wells, this means returning the well to its original state when developed. Original state would relate to both water quality and quantity characteristics as defined when the well had just been developed.

Successful regeneration treatment involves applying a process that removes the clogging or plugging from regions around the borehole and also formations of any biofilms, accumulates, and trapped materials. This is necessary so that the void volume can again become fully available for groundwater flow and not be plugged with biomass and perched bioaccumulated inert materials. Regeneration should therefore get the well back to, or as close to, and sometimes even superior to, when it first went into service. The possibility that the well may perform better than the original specifications is based on the potential for some wells to have had inadequate development and be poorly characterized when the well was first put into service. Delays in bringing a well into service after its original development could mean that the data gathered would have been from a well already suffering from some level of fouling. Characterization after this process has started would give a lower baseline value which means that a successful regeneration is likely to exceed those characteristics as the well returns to its true original performance capabilities. Claims that the well has returned to 140% of its original Q/s may therefore be as much a failure in the accuracy of the original data baseline as a result of highly successful recovery.

The criteria to determine the success of a treatment should include a careful judgment as to the value of the data being used to establish this baseline. Parameters to be considered should extend beyond the Q/s to shifts in the water quality that

occur after the treatment. For the bacterial aspects of the fouling, it should be expected that the activity (aggressivity) should drop and be reflected through longer time lapses after treatment. Minimally time lapses should increase by at least 1 day, with longer delays indicating a more successful the treatment. Taking water samples for this type of testing immediately after treatment is likely to give false data since there would be many bacterial survivors within the water well environment that would tend to give periodically very short time lapses and high aggressivity. It takes 6–8 weeks after treatment for the bacteria in and around a well to stabilize as a part of a reforming biofouling. Until this happens the bacterial activity cannot be reliably measured. As the time lapses become stable, the reaction pattern signatures also become constant indicating that the bacterial growth is now reinfecting the environment in, and around, the well.

Water quality characteristics tend to settle down more quickly than the bacterial activity once the redevelopment has been completed and is often first recognized by the water suddenly shifting from a turbid often colored form to crystal clear water. Clear water indicates that much of the disrupted and dispersed materials have now been removed from the well by the selected treatment process. Water quality parameters (e.g., total organic carbon, phosphate, iron) can decrease in the groundwater flow as it passes the newly forming biomass and then becomes accumulated. This newly forming biomass now acts a natural filter producing water with a much higher quality. This will continue until the biofilms begin to either saturate the voids or slough into the water. Saturation or sloughing can cause sudden and dramatic declines in water quality with products such as iron suddenly appearing in increasing quantities in the product water. Again, the industry has to recognize the unique nature of each well, the need for diligent robust monitoring, and the application of ongoing preventative servicing programs even for wells that appear to present no problems in meeting the designed objectives.

Regeneration treatments are applied to water wells when the specific capacity has dropped catastrophically. What is a catastrophic drop? Water well operators may all have different opinions but most would agree that a significant loss in specific capacity, if it cannot be blamed on the dewatering of the aquifer, means that it is not due to a lack of water in the aquifer but to a lack of ability to get groundwater from the aquifer into the well.

It is well known in the science of chemistry that the use of heat can accelerate the rate of most chemical reactions. It is also a well-known fact in the science of microbiology that microorganisms will only function within a limited temperature range. For example, two features employed in the patented blended chemical heat treatment (BCHT™) process are heat and chemical applications. Here, the chemistry of the treatment (commonly employing nonphosphate biostatic detergent along with significant pH shifts of greater than 4 pH units) has a greater impact when the temperature is elevated by greater than 40°C. At the same time as the heat speeds up the chemistry then the created thermal gradients will kill off or traumatize the vegetative microbial cells with death rates ranging from two to four orders of magnitude. This thermal gradient extends from the borehole until at some distance (such as 6 m or 20 in.), there is a zero impact on the background temperature. Moving up the thermal gradient, there is a period of 1–3 days when the temperature

has been elevated. Close to the borehole such elevations will have effectively traumatized the microbial community with few survivors. Further out along the gradient, the temperature gradient would be less and there could be some microbial activity stimulated by the elevated temperatures and the nutrients that become available from the dead microbial cells. Since the thermal gradient cools down very quickly there can be only a brief window for enhanced growth. If the well has been properly treated then much of the dispersed material would have been removed from the well, minimizing the potential for microbial growth. Claims that these elevated temperatures do stimulate microbial growth has credibility in theory but in practice, the thermal pulse delivered with the heat treatment is too brief to allow an effective growth of microorganisms in the well during the treatment process. It should be recognized that the normal generation times for the indigenous bacteria in a water well has to be measured in days, weeks, or months, while the cooling off period for a well would not normally extend beyond 4 days.

There are a number of basic constraints that should be considered in the collection, handling, and disposal of the discharges that are inevitably a part of a regeneration process. There are some processes that claim minimal discharges since the treatment is so effective that it drives the disrupted materials deep into the formation away from the well. Even if this were to be the case, the action of the extraction, injection, or horizontal well would cause relocations to occur with the potential for covert discharges. There should be open and frank discussions with the responsible regulating agency officials to ensure transparency exists for all of the correct steps that are being taken to regenerate the well with a minimal environmental impact.

Treatment of a well, particularly involving the disruption and removal of the plugging biomass, is an inherent concern. As the biomass is maturing within the well, there is an accumulation of potentially hazardous materials that will be removed from the well during the treatment process. These bioaccumulates can range from recalcitrant organics that have not yet been degraded, daughter products of degradation, or inorganic materials such as cationic species of metals and even radionuclides. In the maturation of the biological plugging (natural filter), these materials gradually accumulate with only a relatively small fraction being periodically released into the water flow through the sheering of the biofilms. Such releases usually take the form of biocolloidal particles moving with the groundwater flow.

Discharges from the successful regeneration of a well can be expected to contain the bulk of these potentially hazardous materials that had been biologically filtered out of the well. For extraction wells, this process would happen as the groundwater moves towards the well. For injection wells, the entrapment would occur as the injected water moves into, and away from the well. It can be expected that accumulates are likely to occur closer to an extraction well and farther away from injection or horizontal wells. Discharges after treatment have different characteristics; with injection and horizontal wells having the most accumulates further from to the well creating a heavier discharge later in the redevelopment. Extraction wells may have disrupted accumulates located at greater distances from the well, along the redox gradient, and may take longer to achieve discharge all of the

disrupted materials. There is always a risk in all wells that some accumulates may be too far back in the formation to be affected by the regeneration treatment but may be released later during restabilization.

6.2 TYPES OF CHEMICALS EMPLOYED IN REGENERATION

Chemical treatment, in a preventive mode, has been a major part of maintenance of well and fluid system performance. At these times, it is important to consider the responsible use of chemicals for preventive maintenance to sustain the well's effectiveness after regeneration. In the industry at large, experience has found that chemical selections for well treatment are often made based on incomplete information or on naturally biased vendor sales literature. This does not mean that the information from commercial literature should be dismissed since many vendors also seek to find effective solutions. It is therefore crucial that personnel engaged in designing the options for the regeneration of extraction, injection, and horizontal wells seek expert advice and review the published literature. This should not be specifically limited to publications written on existing treatments but should also deal with the literature relating to the environment in which the wells are operating. There should also be an evaluation of the features affecting chemical choices particularly with regards to their effectiveness and safety. There are a number of important factors relating to the choice of specific chemicals and these are addressed below.

6.2.1 Issues in Chemical Choices

The listings of chemicals in this section include brief summaries of the chemicals' uses. Major factors include reactivity and costs. Reactivity with constituents of groundwater is an issue in the chemical selection for the regeneration of wells particularly on contaminated sites. Cost is frequently cited as an issue affecting the choices that are made. Three factors affect the market price of chemical products used in well regeneration:

1. Actual product and shipping costs.
2. Premiums for purity and standard certification.
3. Degree of commercial exclusivity (particularly with proprietary products) that exists for the particular product/service.

In terms of effectiveness, it is not necessarily the more expensive chemical that is automatically the most effective. It may actually be a better choice if it is more cost-effective. For example, among the acids, it is the organic-based products that are more expensive than the inorganic acids, primarily because of greater processing costs. However, where there is a severe biofouling, their effectiveness and relative handling safety may outweigh the actual material cost causing advantages of the inorganic acids. It is important to gain an accurate appreciation of the targeted aim for the treatment where selection can be appropriate to the need and not simply a matter of economy.

Project management at groundwater remediation sites should take a long-term approach to cost-effectiveness calculations and consider it as a part of the life cycle cost for the wells. Parallel available research in water supply applications indicates that aggressive preventive maintenance servicing is cost-effective when compared with losses in efficiency, equipment repair, and well failure that would otherwise have occurred. Groundwater plume management adds the factor that the cost of failure to control contaminated groundwater could have serious off-site implications.

6.3 CHEMICAL SELECTION FOR USE IN REGENERATION OF WELLS

Chemicals are all, by the very nature of their purpose, reactive and can pose risks to the operator's skin, mucous membranes, and other body parts. In addition to that, these chemicals can be potentially harmful to the at-site and off-site environments if handled improperly. Care should be used to understand the materials and safety data sheet (MSDS) information. Chemicals should only be handled and applied by trained personnel familiar with their safe use, and who are suitably equipped with the proper respiratory and skin protection if specified for that chemical. No regeneration project should employ personnel or contractors to perform well cleaning who cannot clearly demonstrate competence in these areas. This relates to the use, and thorough understanding of, the potential reactivity between the regeneration chemicals applied and the various contaminants of concern along with other chemicals found to be present on the site.

A general summary of the pertinent factors influencing the selection of chemicals is listed below summarizing chemical purposes, effects, safety, handling, and effectiveness features.

6.3.1 Acids

These are generally used to dissolve hard encrusting materials, including Fe and Mn oxides and carbonate deposits. They are, as a group, very corrosive and safety requirements would dictate the routine use of gloves, splash protection, and respirators, particularly during dispensing and mixing. Of the acids, acetic acid is an excellent biocide and also capable of dispersing biofilms. It is relatively safe to handle and often a major component of many biofouling "enhancers" and brand-name mixtures specified for biofouling. Acetic acid cannot get pH levels down effectively and can rapidly lose its acid power, so supplementation with sulfamic acid is commonly practiced, acidizing down to the <2 pH range. It is recommended that a food grade or good industrial grade (e.g., $>85\%$ acid) should be used. In restrictive sites, the acetic acid can give off odors that would cause complaints and so consideration should be given to this potential irritant in selecting acetic acid. If this odor problem becomes significant then a variation of acetic acid known as glycolic or hydroxyacetic acid can be used. This has the advantage of being odorless, equally effective and viscous, and less susceptible to freezing in the normal working temperature range. In the colder climates, acetic acid may also create problems

through increasing viscosity, as the chemical will freeze at temperatures as high as 10°C–12.8°C. If acetic acid is to be used, then it is recommended that the chemical be stored at normal room temperatures to prevent viscosity increases. It should be noted that this viscosity could severely impair peristaltic pumping efficiency with much lower volumes being delivered over a given period.

6.3.1.1 Acetic Acid

It is a normal by-product of the steel industry as pickling liquor. This creates a quality control problem due to the unacceptable concentrations of cadmium and other impurities often present in industrial grades. These impure grades of acetic acid are NOT RECOMMENDED for the regeneration of wells. Acetic acid can be extremely hazardous to handle. It is recommended that a strong food grade quality acid should be used. This is readily available at 75% strength, in 208-L (55 gal) drums and 45.4–56.8 L (12–15 gal) containers. Odor associated with the use of acetic acid should also be a consideration that could restrict its application in particularly built-up residential areas.

6.3.1.2 Sulfamic Acid

It is a common chemical used in well rehabilitation. It has the advantage of being relatively safe to transport and handle because it is a solid product (dust inhalation should be avoided). It is relatively effective against carbonate scale and also as an acid enhancer for acetic or hydroxyacetic acid. Sulfamic acid is, however, not effective on its own against biofouling or metal oxide encrustations. It is a solid and less aggressive than hydrochloric acid. It takes thorough mixing to dissolve the acid particularly in cold water. However, it is recommended that minimally gloves, a dust mask and goggles should be used when handling this chemical and that adequate ventilation is always provided.

6.3.1.3 Phosphoric Acids

It is another group of the inorganic acids that have been extensively used in the treatment of biofouled wells, pipes, and heat exchangers. While it has been found to be effective particularly in closed environments (e.g., pipes), it is quite hazardous to handle. Full breathing mask and splash protection are required to safely handle this acid and there has to be adequate ventilation. One very significant negative aspect of using phosphoric acid in a well treatment is that it will leave behind phosphate residues. Such residues when in an open porous environment can become a part of feedstock for biomass returning to biofoul the well. Pumping the phosphate solution back out of the well does not remove all of the phosphate, as it will adsorb to minerals such as clays, as well as becoming retained in the more deeply seated surviving biomass. As a result of this retention of phosphorus within the larger treated environment, subsequent growth would be that much faster. As a result, phosphoric acids and other phosphorus-containing products cannot be recommended preventative maintenance servicing and regeneration treatments.

6.3.1.4 Organic Acids

Organic acids, other than the acetic group including glycolic acid, that are sometimes used in well regeneration include oxalic and citric acids. These are useful as chelating agents to disperse particulate material in the water. Oxalic acid is also effective as a primary acidizer in low calcium water (<125 ppm calcium). Its use should be avoided in high calcium water since it is likely to form recalcitrant insoluble precipitates that could cause clogging. Citric acid can be effective but this chemical can be used as a food substrate by many microorganisms and may stimulate post-regeneration biofouling events. It is therefore not recommended. Citric and oxalic acids are typically granular solids and the use of gloves, a dust mask, and goggles with proper ventilation are required.

Acids often create safety concerns in spite of their effectiveness. Some acids, particularly the inorganics are extremely hazardous to handle. Since some are volatile liquids, there is a requirement for respiratory and splash protection. While they remain powerful for removing mineral and inorganic metal oxide scale, they are relatively ineffective against biofouling and some can even be deleterious to stainless steel. Do not mix with chlorine since a reaction in the well could lead to dangerous surface eruptions of hazardous chemicals and chlorine gas. Inhibitors can be used to prevent attack on metal well screens but note that some industrial inhibitors should not be used in potentially potable groundwater (due to toxicity concerns) and the gelatin-based safe inhibitors provide potential nutrients to support future biomass regrowth.

6.3.2 Biocides

These are agents used in the attempt to traumatize, reduce, or eliminate the biomass that is causing plugging and other forms of biofouling in wells. Consistent with water supply well cleaning, reducing the bacterial numbers is not considered a primary objective in the cleaning of wells at contaminated sites, whether for maintenance or rehabilitation. Reducing the bacterial numbers is, however, an important part of the regeneration process in order to extend the longer-termed objectives of sustainable reduction and recoveries from hydraulic impact and other symptoms.

6.3.2.1 Chlorine

Chlorine is, of the biocides, the most widely applied to wells at groundwater remediation sites. Commonly, application is typically as sodium or calcium hypochlorite. In general, it is the sodium hypochlorite form that is preferred because it comes as a liquid and more likely to retain solubility even in high-total dissolved solids solutions. One procedure used to limit and remove biological slimes, growths, and encrustation is termed a "shock" chlorine treatment and standards are available. Well cleaning, maintenance, and regeneration are, however, not so standardized but various methods are available. For example, applied chlorine concentrations can be as high as 500–2000 mg/L. Such high treatment levels are likely to conflict with

some of the chemicals of concern at some sites and the application of such high chlorine dosages should not be considered without careful evaluation of these potential impacts. Chlorine is a powerful oxidant that is well documented to react with organic compounds causing chemical alteration of the compounds to forms that are either more difficult to treat or potentially explosive. In the latter case, there is a risk of violent eruptions of hazardous chemicals at the surface. This latter reactivity, particularly in light of the carcinogenic properties of some chlorinated organic compounds (such as the trihalomethanes), is the basis for increasing regulatory scrutiny of the use of chlorine for all purposes in addition to maintaining potable water safety.

6.3.2.2 Ozone

Ozone derivatives form a group of biocides that are based around the generation of a radically oxidative environment using ozone. Ozone (O_3) is formed by exposure of oxygen O_2 to strong electrical charges. Ozone has to be generated right at the point of application due to its inherent instability. It has been found to be too unstable for safe storage under pressure. Additionally, it cannot be safely transported. As a result of these limitations, ozone is largely impractical for regeneration unless generated at the site as a part of regeneration treatment. Ozone does not yet have a recognized practical application in well-maintenance treatment. While ozone is a powerful bactericide, its short life and inability to penetrate deeply into voids within porous media surrounding wells limits its applicability. It may, however, be used in piping system treatments to repress biological activity.

6.3.2.3 Hydrogen Peroxide

It forms another commonly used group of chemicals that are also bactericidal. Like ozone, aqueous hydrogen peroxide is a powerful disinfectant and oxidant. It has been used with some effectiveness in removing biofouling in wells but there is one major problem. Many bacteria (virtually all aerobic bacteria) produce enzyme systems (such as catalases) that can degrade the peroxide relatively quickly to oxygen and water. The resultant oxygenation associated with the degrading peroxide can actually enhance oxidative microbial growth in, and around, the well. There are a variety of sources of "generic" 50% peroxide mixtures available commercially. It may be used in a piping system treatment to repress biological activity.

6.3.2.4 Potassium Permanganate

It is an oxidizing agent that is commonly used and has some biocidal properties. This chemical is another powerful oxidant that is mostly used in maintaining industrial process systems and in water treatment for relatively uncontaminated water. It is not used as a primary oxidant in well treatments and has been found to be less effective in the dissolution of metals and biofilms which can be effectively accomplished using acids.

6.3.3 Surfactants

These form a further group of chemicals used in regeneration and well servicing. One major chemical group used here is the polyacrylamide and polyelectrolyte-wetting agents. These provide the desired effects of dispersing clogging deposits, biofilms, bacterial growths, and clay/silt build up. It should be remembered that clays can sometimes swell up in the presence of some surfactants and this might create serious posttreatment problems if not addressed in the selection of the surfactant. These compounds are not readily attacked by microorganisms and yet produce major dispersive effects on the biomass and associated elements found to be biofouling the wells. Some surfactants can also act as biocides. For example, the product CB-4 not only acts as a dispersant but also acts as a biocide, particularly when the concentration is raised to 0.5%. There are wide ranges of surfactants that have been used in the industry and important properties should include addition to their effectiveness as biofilm and clog dispersants, they should be relatively recalcitrant (during the treatment) and then degradable (biologically or chemically) without any harmful side effects. These surfactants should be handled, used, and ultimately disposed of according to manufacturer/supplier and MSDS instructions.

6.4 SEQUESTRATION STRATEGIES

In the treatment of wells, another important strategy is to use sequestration. This term can be defined as to isolate, set apart or bound up (such as with a metallic ion) so that it is no longer able to react. These compounds are most properly used in low concentrations in chemical blends. Such acidizing mixtures place biomass and metal oxide components in solutions for convenient removal, once they have been dissolved and dispersed in the water column by the regeneration treatment. Chemicals that can be used as sequestrants include various polyphosphates, pyrophosphates, and polyacrylamide-based compounds. In addition, acetic, glycolic and citric acids and some proprietary acid formulations also have related chelating properties. The phosphate-containing compounds are not recommended for regeneration well treatment. This is because residuals of these compounds (as higher molecular weight, MW, polymers) and the breakdown products (as low-MW pyrophosphate and orthophosphate or phosphate) remain behind in the formation attached to clays and accumulate in the residual biofilms. The presence of an enhanced phosphate resource can subsequently induce enhanced biofilm development, often leading to a faster rate of posttreatment plugging.

6.5 USE OF HEAT

Another important factor in the regeneration of wells, while not chemical, is the use of heat. It is well known that raising the temperature commonly increases the speed of chemical reactions and can, when the temperature is raised enough, be inhibitory, and even lethal to microorganisms. Over the last two decades, the application of heat

as a part of the regeneration process for injection and extraction wells has been increasing. The use of heat usually involves heating the water in the body of the well and its immediate environment. Normally, the well environment is heated to at least 54°C and held at that temperature during the period of treatment that may last from several hours to a couple of days. Treatment fluids can be recirculated through the well from the surface and/or neighboring wells. Chemical solutions are premixed before heating the water for injection into the well. The objective here is to create a thermal zone in, and around, the well for a sufficient time to ensure the successful impact of the chemical treatments. Normally, the temperature is raised to within the range of 54°C–85°C depending upon the structure of the well and the nature of the surrounding media. Heat propagates in, and around, the application source and typically accumulates within the well structure (due to the poor thermal conductivity of soil materials). As the heating time is extended, the thermal shock zone will expand out into the surrounding formations around the well. At the fringes where the temperature rise is only a few degrees, there can be some limited stimulation of microbial activity. Heat can also enhance the circulation and penetration of the chemicals into the formation by inducing a localized convection cell. This application of heat can also cause the swelling and shrinking of clays such as bentonite grouts. Heat alone does not act as an effective regeneration treatment since the chemistry of such a treatment is poor and the end product could be a coagulated plugging that is even more difficult to treat (not unlike boiling an egg!). The best approach to using heat is in a process such as the BCHT (ARCC, Inc., Port Orange, Florida) method with a prudent selection of chemicals.

6.6 CHEMICAL BLEND SELECTION

Typically, no one chemical type will address all encrustation and biofouling removal, suspension, dispersal, and repression needs. Blending approaches can permit more effective removal of multiple problems, or treat a single difficult problem more effectively. The exact blend of chemicals for a particular well-field situation is determined based on an analysis of the needs for removing the plugging and clogging material from the well and must be judged on a case-by-case basis. It needs to be emphasized that all chemical mixtures are far more effective when there is concurrently adequate mechanical mixing and development. This should be specified as a part of the regeneration based upon an adequate analysis of the problem.

As a basic necessity, the Occupational Safety and Health Administration require the MSDS to accompany each container of reactive chemical from point of origin to point of consumption or final disposal. Each person handling each chemical must verify that he/she has read the MSDS, or has had it read to him/her and that he/she understands the precautions necessary. In addition, the MSDS must be on hand to provide guidance in personnel exposure problems, reactivity concerns, neutralization recommendations, and to provide information on basic physical properties (e.g., the relatively high-freezing temperature of organic acids). The MSDS of proprietary chemical blends also permits interpretation of their contents and modes of operation in treatment.

In addition to the concern that all chemicals are handled in keeping with the spirit of the MSDS, there is also a need to look at the product of the treatment—the disposal of the purge water generated by the treatment. Any purge water should be disposed of properly in wastewater treatment facilities or on surfaces such as spreading on soil. The latter will only be allowed in limited circumstances at groundwater remediation sites since the wastewater also contains the contaminants that are being treated. The definition of "properly" will depend on the chemical mixtures, their chemical properties (e.g., pH), and the sensitivity of the treatment or land system. Discharge to any surface waters must be avoided. Phosphate-loaded water discharged to surface waters can cause algal blooms and oxygen depletion, resulting in suffocation of many aquatic animals including fish. Additionally, pH shock can be toxic to aquatic life, and turbidity can cause secondary impacts leading to the loss of oxygen from the waters due to high-biochemical oxygen demands. There are varying regulations in each state governing the issues related to disposal of these fluids if they are not going to stay at the on-site-treatment plant. Consult with the appropriate regulators prior to writing a work plan for each site. Note that many contractors are more familiar with regeneration of water supply wells, where there are no hazards other than the treatment chemicals and the regulations for disposal of the water is less restrictive. Disposal of all liquids must be in accordance with the approved work plan.

6.7 REGENERATION PRACTICES

Below is an example of routine management practices for the regeneration of water wells and the manner in which regeneration can be accomplished starting in 1997 and now a part of the routine practice at the Conoco Billings Refinery. Currently, this refinery operates a groundwater interceptor system (GWIS) consisting of 13 groundwater recovery (extraction) wells, of which seven are also utilized to recover light nonaqueous phase liquid (LNAPL). The GWIS was installed in 1993 and its purpose is to prevent the off-site migration of LNAPL and dissolved constituents, and to recover free product. Overtime, the groundwater recovery wells, associated piping, and near-borehole aquifer have shown a tendency to be susceptible to biofouling, which could result in decreased production if not properly managed.

In 1997, the refinery began experimenting with different well-regeneration techniques to increase performance. The purpose of the well work overs was to reduce or remove any biofouling and mineral precipitates within the area of the recovery well, improving the fluid flow through the aquifer, and into the borehole. Different procedures to accomplish this were tested, including the use of acids and caustics. In 1999, the well-regeneration program was upgraded and expanded based on procedures presented in the educational course *Prevention, Rehabilitation, and Maintenance for Wells: Cause, Effect, and Cure* offered by the National Groundwater Association. In 1999 and subsequently in the annual well work overs consisted of a modified application of a combination of heat, chemicals, and agitation which resulted in significant improvement in well performance. High-pressure steam from the refinery was used as the heat source, the chemicals consisted

of a 15% glacial acetic acid solution plus a small amount of sulfamic acid to lower the pH to approximately 2.0, and a surfactant (1% Arrcsperse CB-4, ARCC, Inc., Port Orange, Florida), and the agitation was applied using a series of jetting, surging, and pumping. The entire screened section of each well was worked using a double surge block that allowed controlled placement of the heat, chemicals, and agitation.

To measure the effectiveness of treatment, step-drawdown test data were collected before and after each work over. The step-drawdown tests consisted of pumping the wells at different flow rates and measuring the drawdown in the casings. Specific capacity (flow in gallons per minute per foot of drawdown) at different flow rates was then calculated from the step-drawdown data. A comparison of the specific capacity data before and after treatment was used to determine the effectiveness of the work overs.

Significant increases in specific capacity have been observed at nearly all wells, especially those that were marginal producers. Increases in specific capacity to over 100% have been obtained, with the average increase being over 50%. The data indicated that well work overs, using a combination of heat, chemicals, and agitation, have been successful in improving fluid flow through the aquifer and into the borehole at the Conoco Billings Refinery. This technique has been used successfully for the past 6 years as part of Conoco's well-maintenance program.

The well-regeneration program generally consisted of sampling and analyses to determine basic groundwater geochemistry and microbiology; conducting pumping tests to determine well yields before and after treatment; and treatment of wells using a combination of heat, chemicals, and agitation.

Health and safety issues are a concern because the nature of the treatment and the chemicals involved, as well as hazards associated with normal refinery operations. The procedures used in the well work over program involved the use of heavy equipment, including drilling rigs and vacuum trucks. The procedures also involved potential exposure to dangerous gases and liquids including high-pressure steam and corrosive acids. The liquids that were introduced into the well had the potential to generate dangerous vapors, which required caution while working around these chemicals. Lastly, when hydrogen sulfide had been detected previously in wells at the refinery, the potential for this gas to be present had to be considered. A health and safety plan for the well work over program was prepared by contractors and reviewed by refinery health and safety staff. This plan included procedures for testing each well for hydrogen sulfide, requirements for personal protective equipment to be worn while handling and injecting chemicals, and general procedures for working around heavy equipment. All contractors and refinery staff on the project reviewed and signed the health and safety plan prior to beginning work. Daily health and safety meetings were also held for the duration of the project.

Groundwater sampling and analyses were used to determine basic geochemistry of the aquifer at the refinery. To determine differences across the refinery, samples were collected from recovery wells in several different areas. These included wells within the oil plume, adjacent to a surface drainage ditch, and down gradient of the site. Tests conducted included field measurements of pH, oxygen–reduction potential, temperature, specific conductance, carbon dioxide, and dissolved oxygen.

Samples were also collected for laboratory analysis of various anions, cations, inorganics, and metals. In addition, samples were collected for biological assays using biological activity reaction tests (BART™ testers; DBI, Regina, Canada).

Step-drawdown pumping tests were conducted both pre- and post-treatment on each of the subject wells to determine the effectiveness of well-regeneration efforts. The step-drawdown tests consisted of pumping the wells at different flow rates and measuring the drawdown in the casings. Specific capacity (flow in gallons per minute per foot of drawdown) at different flow rates was then calculated from the data. A comparison of the specific capacity data before and after treatment was used to determine the effectiveness of the work overs. An increase in specific capacity was indicative of a successful work over.

Each recovery well at the refinery has a different operational pumping rate. The step-drawdown pumping tests were adjusted for each well such that, in general, one step was pumped at a flow rate less than the operational rate, one step was pumped at a rate near the operation rate, and one step was pumped at a rate greater than the operation rate. The step-drawdown tests were conducted sequentially at each well. In completing the tests, the pump was not shutoff between steps but was pumped at a constant flow rate until the well stabilized and then increased to the next flow rate.

Water level and other measurements were collected consistently for each well during the tests. This information included both the time intervals that the data were collected and the duration of each step test. Data were generally collected every minute for the first 10 min, every 2 min for the next 20 min, and then every 5 min for the duration of each test. Occasionally, data were collected at more frequent intervals. The pump tests were typically run for 60–90 min. All information collected were recorded on a table of drawdown versus time. These data were then plotted on graphs using an electronic spreadsheet program with drawdown plotted on the *y*-axis and time plotted on the *x*-axis.

To aid in the regeneration, surging was conducted using a double surge block mounted to a 2-in. diameter pipe suspended by a boom-truck/crane. The surging process was used to loosen and remove fine-grained sediment and biomass from the well screen. Occasionally, when a mineral encrustation layer was present on the inside of the screen, a high pressure jetting tool was used to remove the material. A steam hose routed from the refinery steam system was then adapted to the 2-in. pipe to allow injection of steam while surging to shock bacteria growth and speedup the chemical processes. The chemicals were subsequently added via the surge block to break down and kill the biomass. The well-development procedures were recorded on custom regeneration logs for each recovery well. Notes on the logs included time durations of all treatments, volume and makeup of all chemicals added, and field observations for individual tests and procedures.

The typical regeneration procedure consisted of agitating the entire well-screen interval with the surge block while simultaneously injecting steam. The initial surging activities usually lasted approximately 20 min after which the sediment generated by this process was removed by a vacuum truck. It was not uncommon for up to 0.5 ft of sediment and biomass to accumulate at the bottom of the 6-in. diameter well casing following 20 min of steam surging. The vacuum truck would

then operate for 10–20 min, until all sediment generated had been removed from the well. Typically, this vacuum process also removed most of the water from the borehole. This entire process was then repeated until the well-stopped generating significant amounts of sediment. Usually, two to three treatments were sufficient for treating the well. The recovered fluids, a mixture of sediment, water, and refined petroleum products (both dissolved and separate-phase), were transported to the refinery wastewater treatment system for disposal.

Once the steam surging was completed, chemicals were added to the well. Typically, 100–125 gal of the chemical mixture were added to each well. The volume of chemicals injected was approximately six times the well bore volume, and the goal was to lower the pH of the fluid in the borehole by at least three standard units. The chemicals were added using the surge block tool, with the chemicals being injected over the entire screened interval. Approximately 15 min were required to mix the chemicals, set up equipment, and prepare for the chemical injection. The actual injection of the chemicals required an additional 15 min. After the chemicals were injected, the entire well was surged for at least 5 min, to mix the chemicals with the well bore fluids and push the mixture out into the aquifer. The hot injected chemicals were allowed to remain in each well for a minimum of 12 h. The wells were then treated with the steam/surge/vacuum method described above.

Through this customized approach to groundwater recovery, a well-regeneration technique was developed and proved to be successful at the Conoco Billings Refinery. This approach to regeneration of the extraction wells uses a systematic application of heat, chemicals, and agitation. Significant increases in specific capacity were observed in nearly all wells, especially those that were marginal producers of water prior to rehabilitation. The average percent improvement for these wells from 1999 to 2001 was over 50%, with individual wells showing increases to over 100% of the original specific capacity for that well. Analysis indicates that the work overs affected both the near-borehole aquifer and the screen/borehole area, depending on the well. This data indicate that the work overs were successful in improving fluid flow through the aquifer and into the borehole. Due to the repeated success, this regeneration approach has become an important part of Conoco's recovery well-preventive maintenance program.

7 Spheres of Influence: The Coming and the Going

7.1 INTRODUCTION

Water wells function through drawing groundwater from the aquifer and pumping it out through the well. This water being extracted from the well is creating an impact on the groundwater still resident in the aquifer. In the act of pumping water out of the well (or in the case of an injection well pushing water into the aquifer), a sphere of influence is created in the aquifer. The static water level is affected and declines in the immediate surroundings of the borehole (well) from which the water is being extracted. With a routine production demand on the aquifer, there is a shifting with the static water level around the well being pulled down periodically in harmony with the demand. A sphere of influence now exists around the active well that will change the microbiology and even the hydraulic characteristics. These changes in demand can materially affect both the quality and the quantity of water emerging from that well, but often the changes occur very slowly that the signals indicating degeneration can easily be missed.

In addition to the sphere of influence created by the well itself as it extracts water from the aquifer, wells can also be affected by the spheres of influence from other wells and other activities that affect the groundwater neighborhood around the well. This chapter focuses on the coming and the going of the groundwater towards and away from the well that is the primary sphere of influence of interest. There are a number of events that will be addressed here that relate to interaction between the sphere of influence created by a given well and the surrounding environment. The interactions that will be addressed have some biological significance and relate to:

1. Risks from flooding causing compromises to the well (Section 7.2).
2. Groundwater under the direct influence of surface water (Section 7.3).

7.2 RISKS FROM FLOODING CAUSING COMPROMISE TO WELLS

Risks of flood-inundation of water wells are very real in regions that are low lying and/or are downstream from major surface water discharges. These movements of flood waters, if the waters are not controllable and have exceeded manageable flows,

would now be expected to cause significant problems for downstream wells. In addition to the water flow itself creating risks, there is also the danger from such materials (solid or dissolved) that may also be picked up and moved by the water which could have potentially dire consequences. For example, if human or animal wastes are picked up and carried then these can create serious health risks to the users of downstream water wells. Disinfection of water wells has long been a part of water well practices as a means of reducing health risks and also suppressing the activities of nuisance microorganisms infesting the water well environment. The recent events involving catastrophic flooding have indicated that there is not a standard well disinfection practice that is applicable across the North America. Wells most at risk are those located in lowland areas along streams or drainage ways that may be contaminated with silt, health risk bacteria, and other hazards if floodwater enters down the borehole. Initially, consideration should be given to the concept that all well waters and other water delivery systems in the impacted area are unsafe until tested. It is prudent to drink only water that has been approved by local public health authorities, such as bottled or chlorinated water, until tests show that the potable water supply is again safe.

In the U.S.A., there are unfortunately frequent natural disasters causing many types of human hardship. According to the Federal Emergency Management Agency, as much as 90% of all damage related to natural disasters (excluding droughts) is caused by floods and associated debris flows. Over the 10-year period from 1988 to 1997, floods cost the U.S., on average, $3.7 billion annually with the costs now rising as a result of recent major events such as the disaster caused by Katrina, a force five hurricane, that swept along the gulf coast in the fall of 2005. Such instances of flooding may also threaten the quality of water in private water wells if the flood water directly or indirectly penetrates the wells.

If in doubt about the well water supply being impacted by a flood then it is prudent to follow health department drinking and bathing advisories. Homeowners with water wells should have their water tested if any of the following conditions exist:

1. The well was actually flooded.
2. The well was in close proximity to a flooded area.
3. There has been a change in the water quality (odor or taste).

From the microbiological perspective, it should be remembered that bacteria are native to the water environment, as they are in all other natural environments. Most waterborne bacteria living in wells are harmless, while others cause plugging and corrosion problems and even have the potential to transmit disease. Some bacteria, over time, can cause water quality to deteriorate and/or cause damage to the water well system. Therefore, the presence of certain bacteria in a well water sample should be viewed as a need for further action to determine what corrective measures may be necessary to control the biofouling. There is an unfortunate tendency for the popular press to direct public attention towards the viruses as being the potential major concern in wells particularly after a flood. The reality is more complex in that the microbes (possibly including the viruses and *Escherichia coli*) present in the

flood waters now have to compete or defend against the natural microbes that are already there and dominating the natural filters around the well.

It is just simply not practical to test water directly for every known illness-causing bacteria, virus, and protozoan; therefore, the water is usually tested for a group of indicator bacteria, which can be used as a measure of the sanitary protection of the well and water system. This group of common bacteria, called the "total coliform group," has been a good broad spectrum indicator of sanitary protection for many decades for two very good reasons:

1. Most coliform bacteria do not usually cause disease. However, if and when they do show up in a water test, this indicates that water contamination with fecal contents may have occurred, and therefore the water may be potentially hygienically unsatisfactory or unsafe. Waterborne infectious diseases can be caused by fecal contamination that occurs on the ground surface (in the case of animal waste) or near the ground surface (in the case of septic systems and sewers).
2. Coliform bacteria are good indicators of health risk and are killed by disinfection in the same way that most disease-causing microorganisms would be killed. With few exceptions, if a well is properly disinfected and the coliform bacteria become nondetectable then it may be conjectured that the disease-causing organisms would also have been eliminated.

If tests confirm coliform bacteria are present, water used for drinking and culinary purposes should be boiled for 3 min at a full, rolling boil before use until the source of the bacterial contamination is determined and steps taken to resolve the problem. If *E. coli* bacteria are detected, all uses of the water should be immediately stopped and efforts should be made to locate the source, as well as to remedy the problem.

New wells can sometimes operate for many years, even decades, without showing signs of bacterial contamination, but there are no guarantees since some wells can fail in less than 2 years. An annual well maintenance check, including a bacterial test for biofouling and a health risk assessment, is recommended. It is prudent that the drinking water should also be tested any time that the well water changes in taste, odor, or appearance as well as any times that a well is being serviced. Your state agency or groundwater contractor may be able to provide you with a list of certified laboratories that can test drinking water for bacteria. The person taking your sample should follow the water sample collection procedures for microbiological examination outlined in *Methods for the Examination of Water and Wastewater*. These procedures require refrigeration of a properly drawn sample during shipment to the testing laboratory if it is not possible for the consultant to test the water directly. In addition, commonly more than one sample from a given source is necessary to determine water system quality or biofouling risks due to corrosion or plugging.

If tests confirm coliform bacteria are present, water used for drinking and culinary purposes should be boiled until the source of the bacterial contamination is determined and steps taken to resolve the problem. If *E. coli* bacteria are detected, all

uses of the water should be stopped immediately and efforts should be made to locate the source of the problems to allow effective remedy.

In the case of a flood then there is an emergency need to disinfect and treat the well water. This should be done by a qualified groundwater contractor who will assure proper amounts of disinfecting chemicals are uniformly distributed for the proper amount of time to treat the entire water depth of the well and any associated water system.

Most properly constructed wells can be effectively disinfected using the appropriate techniques, but there are exceptions. If contaminated materials have entered into an inadequately covered well, the well may have to be physically bailed out with special equipment before disinfection can be expected to be successful. In some cases, openings or cracks may have developed in the upper part of the well casing because of damage or corrosion. In other cases, the local soils, porous formations, and rocks may not be adequately filtering percolating water so that bacteria may become more active and grow deeper into the formations than usual. In these instances, special measures will be necessary, possibly including well rehabilitation, reconstruction, or as a last resort the construction of a new well. After treatment, the water supply should always be tested again following disinfection. Your local groundwater contractor can help. You can also contact your nearest public health office for advice. As a routine procedure, all wells inundated by floodwaters should be pumped out and disinfected as soon as possible and then the water tested for safety before being used for drinking, food preparation, or any other domestic needs.

To further ensure a disinfected drinking water supply, well owners should consider a variety of treatment methods that have been shown to be effective. These may include employing chlorination, filtration, and the use of ozone. You should also contact your local or state regulatory official to determine which of the proposed water treatment methods is permitted in your area. Well disinfection will help to control health risks but will not provide protection from pesticides, heavy metals, and other types of contamination. If such contamination is suspected due to the nearness of potential contaminant sources, special treatment is required.

It is important, not just for precautionary protective measures against flooding, to make sure well caps, sanitary seals, and other construction features are periodically inspected and repaired, if necessary, by a qualified groundwater contractor. Water systems should also be properly disinfected as a matter of routine practice following any construction, repair, and maintenance activities. Like other valuable property assets, wells should be regularly and properly maintained. This would also mean ensuring that the grouting (sealing around the outside) of water wells is still effective as a necessary hygienic measure to guard the quality of groundwater supply. Requirements for the grouting of all water wells should be an integral part of a local well construction code. Grouting involves the placement of impermeable material (such as cement or bentonite) in the space between the well casing and the borehole.

One potential contamination source during flooding is old abandoned wells and boreholes that penetrate the same aquifer or breach a zone that would otherwise have been a significant barrier to post-flood contamination. Such abandoned wells and

boreholes that penetrate aquifers or breach a zone need to be effectively decommissioned to reduce this risk. Effective decommissioning means that such wells and boreholes must be properly sealed off to prevent any contamination from entering, circulating within, or leaving such structures. Decommissioning of wells and boreholes should be performed by a qualified groundwater contractor.

Other factors of potential significance are the dangers from electrical shock and also the risks posed to water wells from local sanitary waste or septic treatment systems. If there is any danger of electrical shock from any device that has been flooded then caution would dictate that a certified electrician is contacted to ensure that any risk is corrected. Remember that rubber boots and gloves are not adequate protection from electric shock. In particular, following a flood, do not turn on the pump due to the increased risk of electrical shock and possible additional damage to the well or pump. Septic systems should not be used immediately after floods because of the increased health risk. Also remember that drain fields (such as tile drains) will not work until the underground water table has receded. Another potential concern would be that septic lines may have been broken during the flood causing additional problems.

In cleaning a well impacted by flooding without the additional risks of causing damage, contact a well or pump contractor to remove any build up of mud, silt, and other debris from around the top of the well. Consult the contractor if you suspect that excessive mud, silt, or sediment has entered the well. Remember that the pump may need to be removed for bailers to then extract any build up of mud and silt from the bottom of the well. Dug wells can involve a larger volume of water and attempting to "disinfect" those larger volumes of water is not recommended, but an experienced contractor should be contacted along with regulatory agencies to ensure that adequate protection is put into place.

From the viewpoint of the microbes infesting the water well, flooding has very different implications:

1. Reversal in the direction of water flow, from moving towards the borehole through the aquifer, to a deluge of water of very different quality entering the borehole and moving the other way. These can also enter any fractures and open porous media in the overbearing formations.
2. Quality of the deluge water from above is likely to be very different to the normal groundwater. This deluge water is likely to have a different temperature, clarity, more oxygen and nutrients, and carry a larger burden of microbes that have been swept up into the water during the flood.

A number of reactions to this are likely to occur:

1. Redox front will become unstable and will commonly shift further back from the impacted borehole into the formation driven by the higher oxygen concentrations in the deluge water.
2. There would be a relative "feast" of nutrients in this deluge water that would increase the level of metabolic activity of both the microbes

dwelling down the borehole and some of the alien microbes that are able to adapt to these new conditions.

3. There could be changes in the temperature down in the borehole immediately post-deluge that could cause a short-term rise in temperature. Provided that this rise does not exceed 5°C (9°F) then this impact would be marginal.
4. Salinity may change upwards (if the flood was brackish or seawater) or downwards (if the flood was surface waters from a lake, reservoir, or river). Generally, the microbes down the borehole have a tolerance for changing salt concentration with very significant impacts not occurring until the salt concentration gets up to 6%–8%.
5. There would be a "battle" for living space between the normal microbes living down in the borehole and the alien microbes coming down with the deluge water.

Of these various impacts, the one of most concern relates to the survivability of the alien microbes in the deluge waters and, in particular, those that could cause health problems (pathogenic) in humans. Over a long time period, after most of the alien pathogenic microbes that entered the water well environment with the deluge waters would die off. Some die off would be related to the alien pathogens that are not able to adapt to the environment down the borehole (primarily, temperature is too low, nutrients are too sparse, oxygen too limited, and the competition too tough). *Escherichia coli*, for example, does not have good survivability in a borehole because it has limited ability to adapt, although it can protect itself by pumping out fermentation gases and trying to drop the pH in the immediate environment around its cluster of cells. Normally it could be expected, depending on the intensity of the microbial war down the borehole, that *E. coli* would not survive more than a few days or weeks before the natural processes of self-purification wipe out the cells. Some of the total coliform bacteria are capable of winning the war to gain a slime hold in the borehole by actually crossing to the other side and becoming a part of the communities that can grow down in the borehole. Enteric bacteria (total coliforms) that can do this include some species of *Klebsiella*, *Enterobacter*, and *Serratia*. Another genus of bacteria that can be a problem is *Salmonella*. This includes the species that can cause typhoid, paratyphoid, and many forms of food poisoning. None of these species can easily adapt to the natural environment down a borehole but they can sometimes persist for longer than *E. coli*. A "sister" genus to *Salmonella* called *Citrobacter* can, however, adapt and become a part of the borehole microbial community. All of these genera can be easily detected as total coliform bacteria.

Other pathogenic microbes also have a low survivability in boreholes that have been deluged with flood water but effective disinfection is essential to control these pathogens. For the natural microbes living in, or around, the borehole environment then disinfection can be a challenge that is countered by the microbes tending to follow the redox fronts moving back away from the borehole, and by neutralizing the disinfectants with the slime masses (hydrogels) within which these microbes live. As for viruses that may come in the deluge waters, there is very little evidence that these virus particles survive within this very hostile environment. It would be

expected that such particles would be rapidly entrapped with the slime biomass and these virus particles would be treated as another source of "food" by the native microbes in the well.

7.3 GROUNDWATER UNDER DIRECT INFLUENCE OF SURFACE WATER

Bacteria and other microbes in public water supplies are considered to have the potential to pose immediate and serious health risks to humans. Groundwater under the direct influence of surface water (often referred to as "GWI") forms a part of that risk. This is defined as any water beneath the surface of the ground impacted by the surface waters including

1. Significant occurrences of insects or other macroorganisms, algae, or large-diameter pathogens such as *Giardia lamblia* or
2. Exhibiting significant and relatively rapid shifts in water characteristics such as turbidity, temperature, conductivity, or pH which can be closely correlated to the current weather or surface water conditions.

The term "Under the Direct Influence of Surface Water," therefore, means that the groundwater source is located close enough to nearby surface waters, such as a river or lake, that they can receive direct surface water recharge. This would exist when a portion of the groundwater source's recharge is from surface water, the groundwater source is considered at risk of contamination from pathogens such as *G. lamblia* and bacterial pathogens including viruses, which are not normally found in pristine groundwaters. Since these contaminants are often difficult to detect through traditional testing, the Surface Water Treatment Rule (SWTR) requires most water sources to be treated through a system of disinfection and/or filtration. Local agencies are commonly responsible for defining whether groundwater is under the direct influence of surface water.

Generally, filtration is not required for systems using groundwater under the direct influence of surface water that are able to meet all of the filtration avoidance criteria. Some states, however, automatically require the use of filters for all public water systems that use a groundwater source under the influence of surface water. Check with your local regulations concerning policies regarding the use of filtration.

For systems using a groundwater source there is a need to determine:

1. Source is under the direct influence of surface water; and if it is, then the following.
2. Determine the nature of any intrusion by surface waters.

Sources most likely to be under the direct influence of surface water may be because the infiltration galleries and Ranney wells located near surface waters; in poorly constructed springs and shallow wells located near surface waters. The GWI sources are defined as all infiltration galleries, Ranney wells, springs, and wells less than 50-ft deep, located within 200 ft of surface water. Confirmation of a GWI can

involve a hydrogeologic investigation, or the use of the water quality monitoring (WQM) method to determine whether the potential GWI source is hydraulically connected to nearby surface water. Hydrogeologic investigation requires a licensed geologist while the WQM method does not. Groundwater sources under the direct influence of surface water are considered vulnerable to microbiological contamination. For most affected systems, the rule requires both filtration and disinfection to control this contamination.

The WQM requires 1 year of weekly measurements of temperature and conductivity at both the source and the surface water sites. The surveyor must arrange for statistical analysis of the data to determine if there is a correlation between source measurements and surface water measurements. Independent agencies knowledgeable in the art should check the validity of the analysis. If either the WQM method or a hydrogeologic investigation indicates a hydraulic connection then the source is designated as being in hydraulic connection with surface water.

Microscopic particulate analysis entails the collection of a sample of source water and sending this to a qualified laboratory able to perform microscopic particulate analysis. If certain numbers or types of surface water organisms are found in the groundwater samples, the source is designated to be under the direct influence of surface water. Such sources are classified as GWI and subject to the SWTRs that could include filtration (unless certain source quality and site-specific conditions are met to avoid filtration); or disinfection. In either case, this activity should be performed by qualified personnel. In the event of systems compromised by *G. lamblia*, bacterial pathogens, or viruses then at least a four orders of magnitude reduction in numbers must be achieved (i.e., 99.99% removal or inactivation).

Systems with GWI sources or identified as being "potential" GWI sources have several compliance options to choose from, including:

1. Modify the groundwater source to eliminate direct surface water influence.
2. Develop an alternate approved source (for example, develop a protected groundwater source or purchase from a nearby approved public water system).
3. Attempt to meet the source quality and site-specific criteria to remain unfiltered.
4. Install filtration.

Surface waters generally have lower salt (salinity) content than groundwaters. Additionally, groundwaters usually develop a salinity gradient that increases the salt concentration dissolved in the water with depth. Near-surface groundwaters and surface waters can be defined as containing less than 4000 mg/L (ppm) of total dissolved solids (TDS). Such nonsaline waters are considered to require protection from saline groundwater with the tolerance limit set at 4000 ppm of TDS. Surface waters, for example, used as drilling fluids may come from a variety of sources. It should be noted that while 4000 ppm of TDS can be considered as a critical concentration controlling the growth of many species in the biota (defined as plants and animals), it is not a critical concentration affecting the growth of microorganisms. With these microorganisms that are commonly found in

groundwater, the critical concentrations restricting the range of species able to grow is between 80,000 and 120,000 ppm TDS. Generally, the transitional zone-limiting microbial growth begins to become significant at 40,000 ppm TDS. Specialized microorganisms adapted to high levels of salinity can even still function in saturated concentrations of dissolved solids. It is reasonable to surmise that the gradients of changing salinity are not likely to significantly affect the levels of microbial activity although it will change the composition of species that are active.

In practice, groundwater has the appeal that there are already natural filters in place between the borehole and the source formations. This premise breaks down when there are, for whatever reasons, hydraulic connections between the groundwater and the overburden of surface waters. For such intrusions to become significant, it has to materially change the chemical and biological nature of the groundwater. In the event of the movement of organisms of concern, these natural filtration barriers may be seen as having broken down allowing hydraulic conduits to connect the groundwater directly with incoming surface waters. Natural events such as heavy rainfalls, snow melts, and flooding could each provide conditions when a GWI is likely to occur following one of these events. This is particularly challenged by flooding whether over the grade or through the aquifer and there are a number of methods to control this.

8 Art of Well Development

To be an effective contractor or operator for the treatment of water wells that are loosing their production capacity and/or failing to deliver an acceptable water quality, there are aspects of treatment that can be considered more of an art than a science. Modeling water wells and hydrological impacts may appear to be mathematically precise but if that model does not contain all of the factors affecting the water well then it becomes nothing more than guesswork. Unfortunately pretty well all modeling exercises associated with the geohydrological performance of wells ignores the biological factors that are critical components in plugging and degenerating water quality issues. Groundwater is not a sterile environment and flows through porous media or fractures which will be impacted by the biological activity occurring both on the surfaces and in the flowing water itself.

It should not be considered that well water samples showing significant bacterial activities using the biological activity reaction test (BART) testers have become "contaminated" with these bacteria but rather that the well has become "infested" with these bacteria. Bacteria will always be active to some extent in wells even if at background levels. If there has been a bacterial infestation of a well then the BART testers would show this through a greater level of activity (aggressivity). The reactions observed and the time lapses recorded can be used to measure the nature of (the reactions) and the level of activity (time lapse) bacterial infestation in the well. Contamination relates more to an event that arises from a single source (e.g., plume of hydrocarbons) while an infestation may arise because the environment within, and/or around, the well becomes conducive for bacterial growth. Here the bacteria have come in from the natural environments surrounding the well and are infesting the well because of the more favorable habitat for their activities.

Even Darcy's law is fatally flawed in that it does not consider the impact of microbiological activities on hydrologic characterizations and assumes the mathematically defined flow pathways to be virtually sterile and unimpeded by biomass activities of any type. While mathematical interpretations appear to offer precision, in many cases the models developed using Darcy's law can be impaired by an incomplete understanding of the functional mechanisms, particularly of the microbial activity effects. Decisions based on this law may be become counter-productive causing effort to be directed inappropriately. Some modelers counter these flaws by introducing a randomization method of selection (such as the "Monte Carlo" method) which reduces the model validity to an "intelligent" form of guess work and nothing more.

If it was possible to generate a practical and useful model relating to a water well then the locational shifting and forms of the biomass generating in, and around, the well must be a critical component in the development of a validatable model. Such a model is challenged by the multi-dimensional nature of the shifting focal site (center of growth) of the biomass and the manner in which it impacts within the borehole environment. Two distinctive forms of growth may be expected to occur. The first form of growth would be a vertical irregular cylindrical biomass growth occurring around, and inside, the borehole which would be driven, in part, by the redox front positioning and partly by the flow patterns of the groundwater moving into the well environment. This could be viewed as an upside down slime top hat. The second form of biomass growth would be laterally sited primarily at the water level around the borehole where there would be deployed as shifting redox fronts. These two forms of biomass would interact in complex manners to materially change the movement of groundwater in, and around, the well. Modeling such an event would require the input of critical factors such as:

1. Locations of all of the redox fronts that could be influenced by the well activities.
2. Nutrient and chemical loadings coming from upstream into the well environment.
3. Form of groundwater flow particularly as it flows towards the screen and through the slots.

These events would impact on the location and form of microbiological fouling that would occur within the well and would form a major challenge to models particularly in a manner effectively applicable to wells other than the surrogate well for which the model was designed.

At this time, mathematics, statistics, and computer models dominate hydrogeology with but scant recognition of the interferences that can affect the model when applied to a real biological world situation. Many factors founded in the microbiological milieu related to groundwater can seriously impair the predictive precision considered to be inherent in a model. This comfortable reliance on modeling the future performance of engineered hydrogeological events can initially generate assurance of the prediction.

As the microbial factors are more effectively understood in modeling activities, such considerations can be applied to the engineering design. Such inputs can make the product more precise in the prediction of events by the model and the outcomes in practice. However, that relationship can be impacted gradually by the microbial events as these occur over time Biological factors of significance that could impact modeling criteria can relate to:

1. Plugging within a porous medium which causes reductions in through flows and increases in diversionary flows.
2. Gas formation can cause lateral fractures and foams that can perch and cause diversionary water flow.

3. Bioaccumulation activities lower the concentrations of these chemicals in the downstream water until the accumulation function in the biomass becomes saturated whereupon massive and unpredictable increases in these chemicals will suddenly occur in the downstream water.
4. Synthesis of carbonates can cause occlusive plugging in porous media and reduced transmissivity along fractures.
5. Biocolloidal formations in the water can rapidly change (divert or plug) the flow characteristics of water moving through the engineered structure.
6. Preferential growth at the redox front formed within a designed porous material (e.g., geotextile, sock fabric) could cause losses in the designed permeability leading to failure of the designed modeled system. In other words, if the sock becomes plugged it therefore becomes inefficient in the movement of water.

There remains a need to understand the nature of any well or groundwater system. Some of the more traditional approaches need to be applied to counter the dogma that is created by an over reliance on modeling information. This is particularly applicable to biofouled water wells in that each has some unique form of biofouling. There is a need to include the art of well development which means "listening" to the well, "looking" for the symptoms that the well is exhibiting as a result of biofouling, and "interpreting" information relating to the wells pumping capacity. A true artist of well development looks, listens, and interprets the language of the well when it "speaks." Today, there are very few artists who have the patience and understanding to be able to diagnose a well. Most will turn to the chemical catalogues; trade claims and accountants in order to decide what treatment must, could or might be used.

Listening to the well is a seldom practiced art but one that is very important in the determination of whether a treatment has been effective. Factors involved in listening to the well are found in the sounds that the water in the well makes when it is being pumped or surged, and secondly, the sounds made by the pumps and pipes may also indicate stress and potential failure. Listening has to also include paying attention to the local well operator who works with the well on a routine basis. They will often see subtle changes in water flows and quality that may have a major potential impact on the manner in which the well could be successfully treated.

Looking is also important at the well site. Tell-tale signs of a failing well may be littered all around the site from recently grown slimes to well-established encrustations, fouling pipes, and plugged impellers. Records of the pumping capacity for the well are as important as looking at the blood pressure records is to a doctor concerned about the health of a patient. If the pumping capacity is falling then so would the Q/s. There is a strong possibility that the well is edging into a foul fail state. Looking at these factors can help to define the treatment that is most likely to be effective at rehabilitating the well.

Dreams generate schemes that then could lead to resolution of problems. For fouling water wells, the dream would be to have some sort of device that you could aim down at the ground and it would take a digital picture of the biofouling

biomass around the well. Such information would now allow the treatment to become more focused through the use of surge block to provide local applications of the treatment to the sites identified. Also the use of satellite wells can introduce treatment strategies and recover the products of the treatment. At this time there is no such device and any attempt has to rely on potentially corrupt secondary and tertiary data. For example, monitoring wells and piezometers may function to determine static water levels but they can become severely biofouled, particularly where fabric or geotextile socks have been used to prevent the admission of fines into the borehole.

At this time the best "picture" of the health of a well can be estimated by looking at the historical Q/s (specific capacity) for the well and BART data that would indicate which bacteria are dominating in the infestation of the well. Using the zones of interrogation projections (ZIP) on the well it is also possible to determine through sequenced pump samples, the location of these infestations. If a treatment has been successful then the ZIP taken at least 6 weeks after the treatment should show that suppression in the activities of these biofouling bacteria has occurred. As for the digital dream of recording the location of the biomass cloistered around the well, that remains a dream but not so distant. We do know that the biomass has a love for accumulating metals that makes the mass denser and more determinable. Such a biomass, particularly with a significant iron (metal) content, now becomes much more resolvable using reflective electromagnetic radiation devices. Research is ongoing in this field and maybe one day it will be possible to directly record the location of these growths. Until then there is Q/s and ZIP which will give an effective indication of the state of the health of the well.

In medicine, the common treatment is a pill that is swallowed or a liquid that may be injected. Both have a defined quantity of active ingredient that is set to the weight, gender and severity of the dysfunction in the patient. Water wells do not have a set weight, gender and the severity of the dysfunction is often difficult to determine. One feature that can be determined is the well volume which is set by the volume of water inside the borehole. Treating water wells uses the basic unit for treatment commonly set in well volumes. One well volume would mean that the amount of treatment being applied would be effective for just the water volume within the borehole. Very often, particularly after video camera logging where growths are observed intensely inside the borehole then the treatment becomes similarly focused on that one well volume. Accountants like that amount because clearly the well does need treatment and it makes sense to clean out the well and recover the pumping capacity. One well volume appears from the video camera logging to account for the biomass within the borehole but does not indicate the nature of any biomass beyond the field of view.

Video camera logging can illustrate in a very direct manner the dysfunctioning of the borehole but not of the water well itself. Some well drillers are loath to see video camera logging since this may reveal errors in the construction of the well. What the video camera logging does not address is activities associated with the well that extend significant distances away from the slots, perforations or fractures back out into the formation material. Here the sphere of influence created by an active

well extends outwards into the aquifer and two events are occurring here that can significantly affect the functioning of the well are:

1. Water moves towards the borehole created by pumping demands and fines (sands, silts, and clays) may become perched in the formation causing clogging of the throats.
2. Water moves from the more reductive to the oxidative environments closer to the borehole then redox fronts form where biomass would then tend to grow and cause plugging.

Occasionally, the clogging created by the fines can occur at the same location where the plugging biomass is growing. Here, there would now be the accumulation of fines within the biomass. This would mean that when an effective regeneration treatment of a well is achieved there may be a secondary "flood" of fines into the well. This is particularly a problem for the sands that can build up in the borehole. In such circumstances the sudden appearance of large amounts of sand in the well is not a signal of failure but a signal of success. After all, the sand in the well can be removed by vigorous surging action to take it out of the well where it is no longer a threat to the production capacity of the well.

One challenge is the determination of the amount of formation material around the well that would be treated at the same time as the borehole is being treated. One well volume would be expected to treat the water just in the borehole and perhaps the slots, perforations, or fractures in intimate contact with the borehole but not more. One bore well volume is therefore only suitable when there is to be a preventative servicing applied or regeneration where it is known that the biofouling is limited to the borehole itself. If the biofouling extends back into the porous media and formation material around the borehole then greater than one well volume would need to be applied.

One of the major problems in the regeneration of water wells is the risk of complacency for water wells which commonly function over years and decades and any changes are usually subtle. Past practices have been to abandon and replace. This creates economic activity and an assurance that the new well will now function just like the old well when it was first developed. This is not always the case particularly for those who do not recognize that the immediate environment around water wells abandoned because of biofouling is heavily infested with the microbes associated with the plugging of the original well. Drilling a new well close to the abandoned well virtually guarantees that the new well will be taken over by the microbes left behind from the abandoned well.

When installed within a zone already biofouled, the life span of the new well will be negatively impacted and a shorter operating life can be an almost inevitable consequence. While that might be good for consulting engineers and well drillers, it is not good for the economical and sustainable operation of the new well. Positioning a new well within a well field known to include historical evidence of biofouling is another art. There needs to be minimum distances between old wells and the new wells. Basic guidelines would include using the maximum distance of

convenience, moving upstream within the aquifer, examining the Q/s and ZIP data along with any other information to determine the best location which will almost inevitably not be the most economical (from the accountants point of view) or the most convenient (from the plumbers and planners points of views). That is why the art of well development is so important since it is essential to build and maintain well fields that offer confidence that a sufficient volume of water will be produced of an acceptable quality to comfortably meet demands.

9 Illustrated Guide for Groundwater Microbiology

It is sometimes thought that a picture is worth a thousand words. This chapter is formed by a collection of 79 figures that relate to some aspects of groundwater microbiology. The original plan was to include these figures directly into the chapters and appendices relevant to the text. However, it became very apparent that many of these figures could actually be related to more than one chapter which makes cross-referencing a challenge. To resolve these problems, all of the figures have been placed in this chapter in an order that follows roughly the book's first eight chapters. After developing the book, it was clear that this chapter containing the graphics is essentially independent of the rest of the book, and therefore should be considered as a separate source of information that can augment the information presented in the rest of the book. It is therefore recommended that this chapter be read as an adjunct to the rest of the book and hopefully clarifies some of the concepts being presented in the text. Cross-references to other parts of the text can be found in the index of the book and also in the contents pages at the front of the book.

9.1 NATURAL FILTERS AROUND WATER WELLS

Water wells are commonly viewed as simply a vertical tube going down into the ground with some means (such as slots or perforations) that do allow water to enter into the borehole. Figure 9.1 is a vertical section down through an extraction well (W) that has slotted screen (S) covering the bottom section of the casing. The borehole in the well has a static water level (SWL) where the water level becomes stable when the well is not being pumped. A flow in the groundwater flow when the well is being pumped is indicated by arrows with (1) showing the flow paths that the groundwater has taken through the aquifer and (2) then upon entering the environment around the well. This well is shown with three zones of natural with the largest being further out from the well (3). Next and more intimately surrounding the well is the one with more microbiological activity (medium shade, 4). At the well, there is the final natural filter which is commonly associated with the slotted screen (5) and usually is the site where the redox front forms and the biomass is greatest. This final filter may extend upwards into the water column. Therefore, Figure 9.1 exhibits three distinct natural filters as impacting the groundwater as it flows into the well through zones 3, 4, and 5.

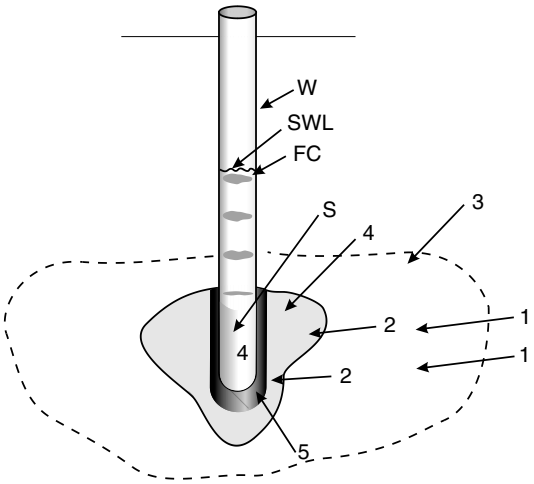

FIGURE 9.1 Diagrammatic presentation of the functioning of natural filters around water wells.

Commonly, these natural filters form very quickly after the well has been developed and will affect the manner in which water flows into the well. For example, when a biofilm first forms contiguously over the surface of the pack material then water flow will meet much less resistance to flow and will therefore move faster. As the biomass expands and enters an encrustation state then this will offer move resistance to flow and the groundwater flow may become slower. Additionally, it is to be expected that floating colloids will be observed growing in lateral regions in the water column below the static water level.

9.2 CHANGES IN POSITION OF BIOMASS AROUND WELLS

Not only does the biomass form distinctive natural filters around the water well as it matures (and possibly biofouls) but also the biomass is constantly moving. Therefore, Figure 9.2 shows the historical development of a water well (vertical discontinuous axis). Maturation of the well is defined as numbered five events occurring over an undefined period of time with the location of the biomass (horizontal axis as distance away from the well slots or perforations). With the maturation of the well moving forwards to eventual abandonment, the center of growth activity for the biomass also shifts. This movement of the focal center of the biomass is shown by a circle with quadrant. This indicates the focal site for the center of the biomass activity relative to the distance from the well is shown as a distorted bell curve. There are five descending graphs which show the status of the well from newly developed (top, 1) to functional to biologically impact (2, 3, and 4) through to abandonment (bottom, 5). Slots/perforation positions are shown as vertical dashed lines in Figure 9.2. It should be noted that the center of growth for

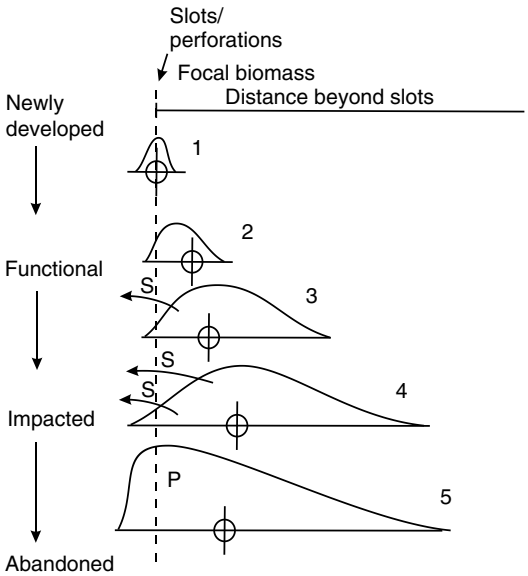

FIGURE 9.2 Phase diagram showing the manner in which the well water quality becomes impacted by the locations of the biomass forming the natural filters.

the biomass moves away from the well during the history of operation and will occupy a greater volume of the porous media surrounding the well leading to reduced transmissivity and reduced specific capacity (Q/s).

Figure 9.2 illustrates the nature of the manner in which biomass is not very much a static entity occurring only at one site associated with the water well but is mobile (fundamentally following the redox front, the source of any nutrients inputs, and the nature of the water flow patterns around the well). In "treating" this biomass through regeneration or preventative servicing methods as the two treatment options, the location of that biomass becomes critical. Some contractors core around the infested water well to determine the most likely sites for this biomass before conducting a treatment.

Essentially during the life span of water wells, it can be expected that initially the biomass would move away from the borehole into the formation. As the well matures then the biomass may trend to move back towards the well. This means that any treatment to control the biofouling should consider the location of this biomass. For example, during maturation, it may be expected that any treatment would have to involve a greater zone of treatment.

9.3 GROWTH OF BIOMASS AND IMPACT ON WELL CHARACTERISTICS

As the biomass grows in and around the water well, then there are a series of impacts on the ability of that well to produce water as the biomass first forms

the natural filters, expands, and then finally plugs the well. In Figure 9.3, the effects of biomass growth and location are summarized into four critical factors (given as four separate graphs). Each graph is given as an *x*, *y* plots, where *x* is consistently the timescale for the aging of the well and *y* is the value for the parameter being presented. Specific capacity (Q/s) is presented as the top graph with the *y*-value being given as a percentage of the original Q/s value when the well was first developed. Microbial loading is defined in the next graph by the relative microbiological activity detected using such methods as adenosine triphosphate (ATP) or time-lapse using the HAB-BART system. This activity is shown on a percentile scale from 0 (none) to 100 (very active). In the third graph down from the top, the *y*-axis now shows the total iron being released in the produced water using a scale from 0 to 10 mg Fe/L. There is going to be changes in the relative occurrences of ferric (Fe^{3+}) and ferrous (Fe^{2+}) forms of iron is shown as dashed lines, respectively. In this example, conditions within the water well environment move from an oxidative dominance to a reductive dominance causing shifting from Fe^{3+} to Fe^{2+}. In the last graph, a relative scaling (0–100) is used to reflect the appearance of other metals in the produced water over the same time periods given in the above *x*-axis. As the biomass becomes saturated with bioaccumulated metals then these excesses begin to slough from the biomass into the suspended bioparticulates and appear in the product water.

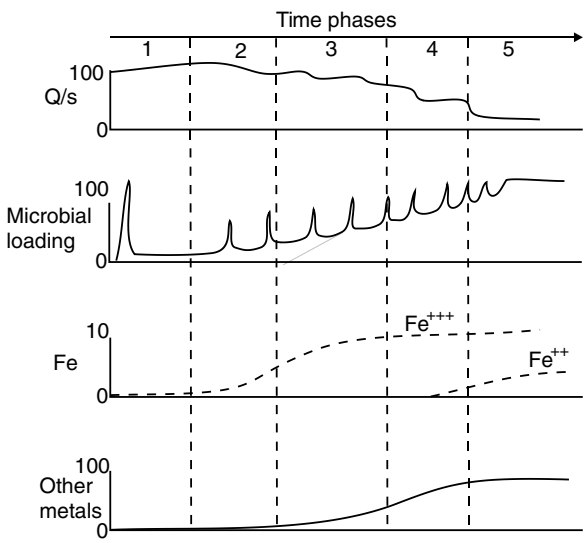

FIGURE 9.3 Chronologically presented diagrams illustrating the manner in which the biomass activity within the natural filters can impact the water quality and productivity of water from a biofouling well.

9.4 GROUNDWATER FLOW THROUGH FOUR BIOFOULING WATER WELLS

Horizontal plan view of four wells (open circles A, B, C, and D) that are each surrounded by a biomass-forming natural filters (dashed circles) in which the groundwater flow becomes contained by the most viscid waters found surrounding the biomass. Viscosity is created by the biocolloids generated by the biomass through the production of polymers by the microorganisms within the biomass. Movement of groundwater flow is shown as short arrows indicating the local direction of flow (from the top of the diagram). In Figure 9.4, the filters that have formed around well B are now significantly affecting water moving towards wells C and D downstream from well B. Well A does not have such a major impact on flow rates. Resistance of the biomass clustered around these wells collectively affects the direction and velocity of groundwater flow through the well field. Thus the natural generation of biomass surrounding each of the four wells affects not only the direction of flow but also the quality of that water. For example, water that has flowed past well D is more likely to be impacted by sloughing biomass from all four of the wells in the well field. There are therefore a number of forensic evaluations that have to be undertaken since it is quite possible that each of the wells will be

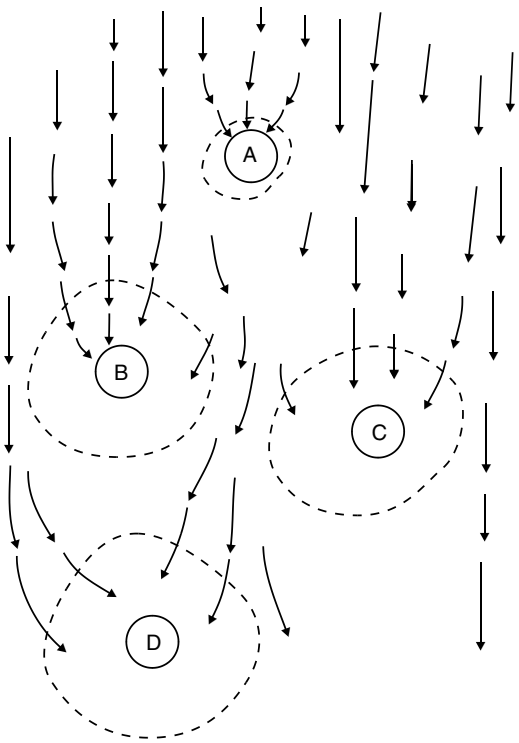

FIGURE 9.4 Illustration of the manner in which biomass positioning around the water well would be impacted by groundwater flows.

affected differently in terms of not only the production flows (downstream wells are more likely to be suffering biofouling impacts) but also the qualities (iron concentrations, oxidation–reduction potential (ORP), turbidity, and bacteriological counts). By examining the data from the various wells within a common field it is possible, with knowledge of the production from these wells overtime, to project the likely sites for the biofouling activities around these wells. In the "Sherlock Holmes" mode imagine that you are looking at the clues for a heinous crime that was plugging up the wells and losing water production and quality from the wells. Examining the data thus allows a visualization of the biofouling levels in the wells. Of all the characteristics, it is the ORP that is perhaps the first clue to examine.

9.5 DIAGRAMMATIC PRESENTATION OF BACTERIAL CELL

Microorganisms are the big villains living in the biomass biofouling water wells. Of the microbes involved in the development of the biomass, it is the bacterial cells that tend to dominate. This is because of the very adaptable nature of the bacterial communities allows them to grow in the presence of oxygen (aerobes), in the absence of oxygen (anaerobes), and even where the oxygen may be present or absent (facultative anaerobes). An ORP measurement of the water correctly taken will identify which of these bacteria are likely to be dominant. For the other microorganisms, such as the fungi (molds), protozoa, micro-algae, and viruses, there are limitations. For the fungi they require oxygen and not so much water, they are found in the semi-saturated zones above the water table. Protozoa are like very simple animal cells that feed (like carnivores) on other microbes but only in the presence of oxygen. That limits their presence in the biomass. Micro-algae are capable of growing under anaerobic (oxygen-free) conditions but need light to allow photosynthesis. If there is no light down the water well then there is no algal growth. Viruses are total cell parasites and have to infect specific living cells in order to replicate. The only group known to infest water wells is the bacteriophage that selectively infects some bacteria. Other viruses may survive in the groundwater and biomass but run the distinct risk of being accumulated and degraded by members of the biomass.

Typically, the bacterial cell has a simple rod shape with very little in the manner of internal organs. In Figure 9.5, the cell is shown bearing fimbrae (S, spikes) out of the cell wall (E, envelope). For clarity, the lower two-thirds of the cells is opened to show nucleus (N, black twists), ribosomes (R, black ellipsoids), and storage granules (SG, open circles). A single flagellum (F) is shown extending from the base of the cell through the cell wall. It is the flagellum that allows the bacterial cells to "swim" through the water.

Commonly, the bacterial cells are clustered within the polymers forming much of the biomass and any treatment has to get through this to the cells in order to kill the cells. Challenges are created by the chemicals needing to be employed to break down the polymers and then destroy the cells. Any treatment therefore has to shock, disrupt, and then disperse the biomass destroying the bacteria.

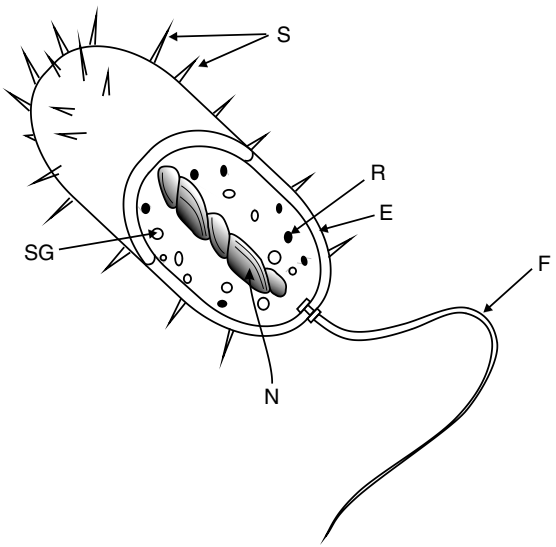

FIGURE 9.5 Diagrammatic presentation of the bacterial cell as the dominant microorganism in the biofouling biomass.

9.6 CROSS SECTION OF ATTACHED BIOMASS

Most (commonly $>85\%$) of the biomass growing around a water well is actually attached to surfaces, such as the slots, perforations, pack, and formation material (gravel pack and fractures). As the biomass attaches to the surfaces, it naturally grows into the voids through which the groundwater is flowing. Thus as the biomass expands to greater volumes, there is a shrinking of the void volume and throats (openings) through which the groundwater flows towards the well or on downstream from the well. The bulk of the biomass is biocolloidal in nature and this can have a major impact on flows. Such biocolloids can effectively affect the patterns of water flow movement around the impacted well.

In Figure 9.6, the attached biomass is shown as a vertical section through the bacterial slime (biofilm) attached to the surface (S). Water flow moves down through conduits (water flow shown as arrows) into and through the slimes. Various microbial communities are indicated in the slime as ribbons (G, *Gallionella*), tubes (SB, sheathed bacteria), black circles (microbial consortial clusters, CC), and triangles (attached microbial biomass, ABM, attaching directly to the underpinning surface shown by lines). Access channels (AC) allow limited divert water flow into the biomass. The slime (glycocalyx) is outlined as a light grey mass.

In the development of a treatment strategy, then one of the prime factors has to be to destroy these slimes and leave the surfaces to which the biomass was attached pristine again. This would now allow the groundwater flow to again move without being impeded by the attached slimes. However, cleaned pristine surfaces are naturally attractive to microorganisms that are capable of attaching.

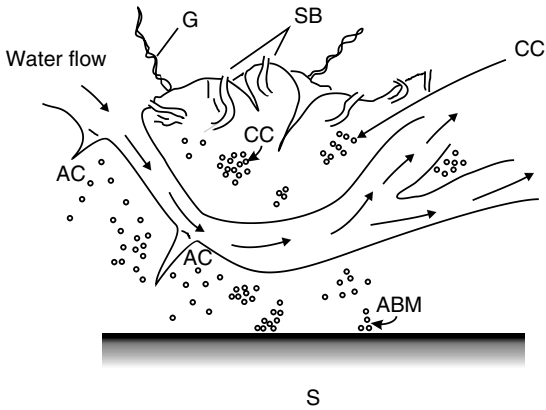

FIGURE 9.6 Cross section of the microbial slime.

If this happens then the whole biofouling process will again be repeated. The bottom line is that even if you can treat and clean the well so that it returns to its original developed values, attachment, and refouling of these pristine surfaces is almost inevitability going to happen. Ongoing treatments need to be planned for by an effective preventative servicing of the well on a regular basis and regenerative treatments as needed. This would then make the water well more sustainable.

9.7 MAJOR FORMS OF BIOFOULING IN WATER WELLS

Biofouling can involve many different forms of growth commonly dominated by bacteria, but with fungi involved along with protozoa on the oxidative side of the redox fronts. Six of the most common forms (as vertical sections) of microbial growth in groundwater are depicted in two columns and three rows. In Figure 9.7, upper left (1) depicts a biofilm that commonly has a smooth (S) surface and a gelatinous structure (G). Upper right (2) depicts a condition when the biofilm now matures into an encrustation with a hardened (HR) crust coating and dense accumulates inside (A). Middle left (3) shows the form of a nodule. Nodules are knob-like structures that can often be observed growing inside mild steel. These usually have a hardened smooth crust coating (HS) with corrosive activities (C) often forming perforation pittings occurring on the underpinning surfaces beneath the nodule. Middle right (4) depicts a floating biocolloid (BC) with an ill-defined edge (IE) suspended in the water (waves). These can become so numerous that the water becomes gel-like and can even have particles locked within the gelled water. Lower left (5) shows suspended silt particles as accumulates within a hardened smooth shell (HS) with the ability to control density (CD) and so float within the water. Lower right (6) depicts planktonic microbiological activity within the water. Here, the motile microbes (MM) are able to move in distinct directions (DD) through a swimming motion commonly using flagella. These are

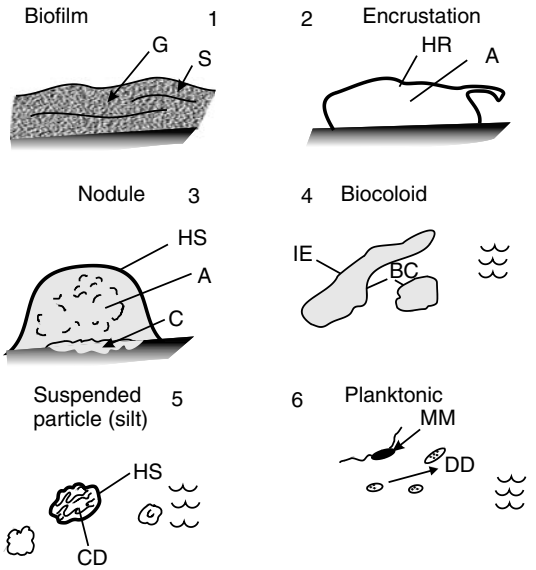

FIGURE 9.7 Illustration of the different forms of microbial growths in water wells.

the whip-like extensions out of the cells which are able to propel the cell through the water.

Each of these different structures presents a different set of challenges in the treatment and management of these events. Some of the attached types of growth (1, 2, and 4) can sometimes be physically removed by disruption (DR; e.g., brushing), while others need disrupted chemical destruction that may facilitated through the use of heat.

9.8 FORMS OF COLIFORM BACTERIA

Regulations protecting water supplies from hygiene or health risk rely heavily on the presence or absence of coliform bacteria in the water. Regulators frequently emphasize a zero tolerance to the presence of total- or fecal-coliform bacterial cells in a 100-mL water sample (WS). Figure 9.8 includes five different forms of coliform bacterial cells. These are depicted to illustrate major features and these are not to scale. On average, the cells are measured in microns (thousandths of a millimeter) and normally are found in the range of 0.5–4.0 μm.

Upper left are two cells of *Escherichia coli* (also commonly called *E. coli*) showing the cells to be rod shaped (RS) with the right-hand cells possessing flagella (F). These are the high-risk species and are commonly found in human feces. It is some of these species (a minority) that can be pathogenic in humans. Additionally and perhaps more importantly, the presence of these fecal coliforms also shows that there is an increased risk of other human bacterial pathogens being carried in

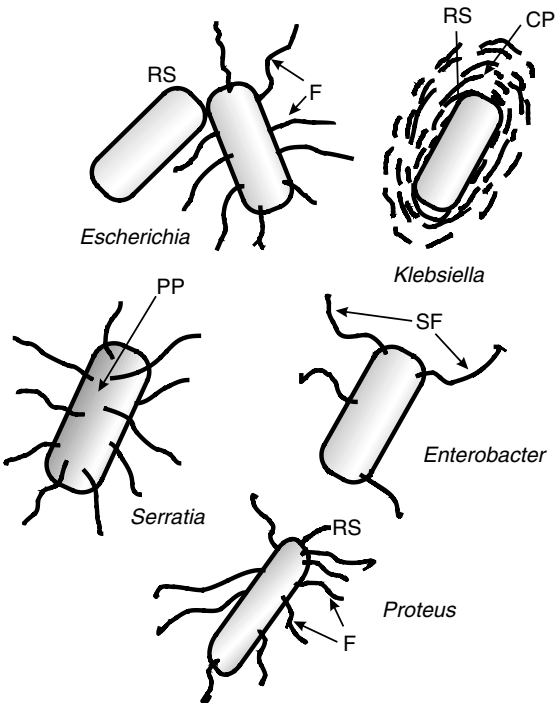

FIGURE 9.8 Forms of coliform bacteria.

the same water. *Escherichia coli* presence therefore portends a significant potential threat (risk) in the water to human health.

Upper right is a cell of *Klebsiella* species showing the thick capsule (CP) that can form around the rod shaped cell (RS). Middle right is a RS cell belonging to *Enterobacter* species with just a few sparse flagella (SF) attached. At the bottom of the figure is a RS cell belonging to the genus *Proteus* showing dense flagella (F) covering the cell. Middle left is a RS cell of *Serratia* that is shaded to represent the pink-red pigment (PP) that these bacteria normally possess.

All of these species of (enteric) bacteria form a part of the larger group of coliform bacteria referred to as the "total coliform bacteria." This group also includes some of the enteric bacteria that can grow within the environment such as in the biomass around water wells. Zero tolerance for total coliform bacteria provides an additional level of protection but a significant number of water wells do normally contain some species of total coliform bacteria.

9.9 FORMS OF IRON-RELATED BACTERIA

Iron bacteria have long been considered as one of the principal problems in water wells. This is partly because these bacteria are able to accumulate in different ways ferric forms of iron. Ferric is an oxidized form of iron that has a distinctive orange,

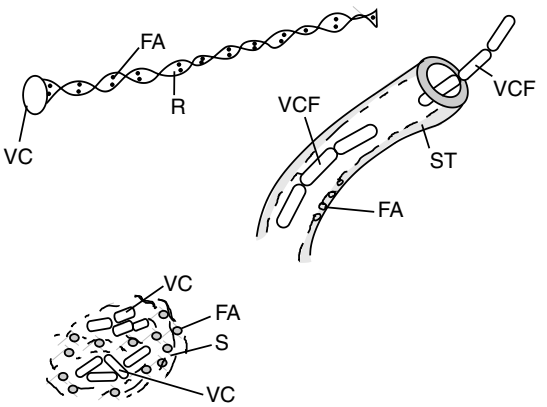

FIGURE 9.9 Forms of iron-related bacteria.

red, or brown range of colors depending upon the concentration of ferric-iron in the ochre. For a long time, it was thought that these ochres were geochemical in origin. With the advent of microbiology, it has been found that these ochres are actually different forms of microbiological growth. Here, the microbes use combinations of passive accumulation to active exploitation of the iron sometimes converting it from ferrous soluble to the ferric insoluble forms.

In Figure 9.9, three diagrams depict the major forms of iron-related bacteria (IRB) including *Gallionella*, the sheathed IRB, and an encrustation of IRB.

Gallionella is one iron bacteria that does appear to be able to exploit the oxidation of ferrous- to ferric-iron as an energy source. Here the ferric accumulates (FA) is extruded as a commonly ribbon-like (R) tail. The other iron bacteria do not appear to be able to exploit to accumulate the ferric forms of iron as complex crystalline structures that then provide protection to the iron bacteria living within these ferric growths. The growths include viable cells (VC) or filaments (F) that can be living in sheaths (ST) or in hardened shells (S) to resemble encrustations or rusticles. These types of growth can be considered to form a type of clogging and thought to be geochemical in origin. In reality these types of iron-rich growths originate from the growth of iron bacteria.

Iron bacteria are a complex term which covers all of the bacteria able to accumulate ferric forms of iron. It was proposed to use the more embracing term "IRB" since this would cover all of the bacteria able to accumulate ferric-iron and also ferrous iron depending upon the reduction–oxidation potential.

9.10 BIOCOLLOIDS

Water that is rich in microbiological activity is likely to contain also polymers as a product of the growth of biofilms attached to surfaces. These polymers do have the ability to bind and hold water in a gel-like form that means the physical characteristics of the water will change. For example, water entrapped within biocolloidal structures will have much less ability to flow as freely as liquid water. This

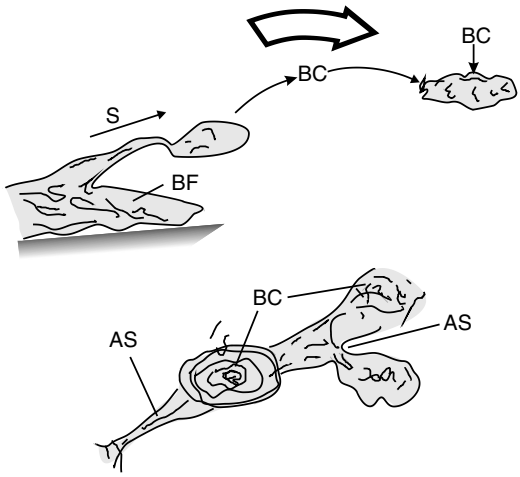

FIGURE 9.10 Diagrammatic presentation of the nature and origins of biocolloids.

biocolloidal water therefore creates changes in not only the state of the water but also affects the rate at which the water can flow particularly through porous media. Additionally, these BCs can also increase the ability for the accumulation and eventual degradation of organics.

In Figure 9.10, upper diagram shows the manner in which BCs can sheer away from a biofilms (BF, slime) which can then be subject to sheering (shown by arrows). Here the sheered particles are biocolloidal (BC) and can become suspended in the water. In the lower diagram, biocolloids are shown suspended in the water but interconnected by attachment strands (AS). On the occasion, these AC structures can totally gel up the water so that it shows little or no free flow. Essentially the polymers holding the bound water can sometimes form a spider's web of strands that effectively then binds the water.

Treating groundwater that has high-biocolloidal contents creates a need to employ some chemistry that would specifically destroy these BCs. The most effective chemical treatment for this is the low phosphorus detergents that have the ability to destroy these polymers. Advantages in the chemistry of the detergent are the presence of some biostatic agents that would prevent the microorganisms from reforming these polymers after the treatment has been completed.

9.11 SULFATE-REDUCING BACTERIA

In the history of water well development and exploitation, it was the sulfate-reducing bacteria (SRB) that first got the attention of industry (Figure 9.11). Events that developed this early interest was a combination of corrosion of steels associated with hydrogen sulfide that then causes electrolytically initiated corrosion; the generation of noxious black slime stinking with "rotten egg" odors; and the reduction in water quality associated with the presence of black sulfides in the water.

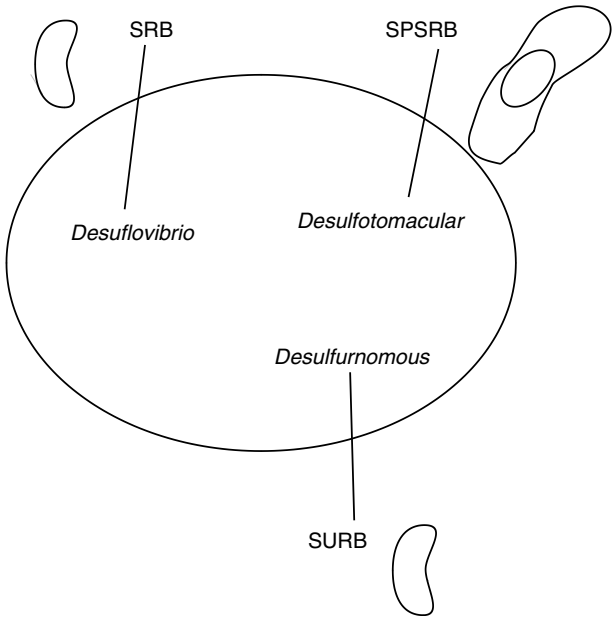

FIGURE 9.11 Geographical location of the sulfate-reducing bacteria (collectively known as the sulfate-reducing bacteria) on the bacterial atlas. Arrows depict the movement of spores through the groundwater flow.

Initially, it was thought that all of these phenomena related to geochemical problems and did not involve any major bacteriological factor. Today the bacteria utilizing sulfate and generating hydrogen sulfide (with various secondary impacts) are categorized into three major groups. Two of these bacterial groups do reduce sulfate to hydrogen sulfide and differ in that one group (SRB) includes two genera. One has the ability to make spores (survival cells) while the other does not. The second group does not reduce sulfate but reduces elemental sulfur. In all cases, the end product of activity is the production of hydrogen sulfide, the primary cause of corrosion, odors, and black-slime problems.

There are three principle genera of SRB that are shown on the atlas for Section 9.7. By each designation is the typical cell shape observed for that form of SRB. There are three major types: sulfate-reducing bacteria *Desulfovibrio* (SORB); sulfur-reducing bacteria (SURB); *Desulfurenomonas* and spore-forming (SPSRB), *Desulfotomaculum*.

In practice, these three groups of SRB are found in very different circumstances. SURB are the most specialized since these rely upon the reduction of elemental sulfur and so is not found in the habitats unless elementary sulfur is present. SPSRB are a challenging variety of the SRB that have the advantage in being able to survive harsh environments by going into the sporing stage. In this stage, the cell now protected in a spore state becomes much more resistant to treatments which it can now survive and then germinate and grow as a regular cell.

9.12 FUNGI (MOLDS)

Higher up in the formation material generally just above the static water level, there is commonly a dominance of molds (fungi) usually as a layer in the semi-saturated zone where oxygen is permeating down and the water is permeating upwards bearing the organics. Of all the fungi, it is the mushroom *Agaricus campestris* that is the most well known. It is interesting that the head (button) of these mushrooms only represents 5% of the fungal biomass. The other 95% is down there feasting in the semi-saturated zone just above the static water level where there is oxygen. These microbes are colonizers and put out their growths in all directions where there is oxygen and water. These growths (called mycelia) generate thread-like bodies that sometimes resemble flattened loosely woven textile with very fluffy sides. As these molds grow outwards often in a lateral manner then the cells in the middle begin to produce spores. Looking at some old dry wall that is infested with black mold gives a good picture of this growth. Where there is the black dust on the board then it is here that the molds are producing spores. Just like with the mushrooms, most of the growth is now out of sight in the dry wall where it is growing on the moisture in the dry wall (that normally should not be there) and oxygen from the air.

Theoretical diagrams of the typical growth of mold growth (upper) are shown in Figure 9.12 and the focus site where mold biomass tends to be the most active as a mycelium.

Mold growths are therefore commonly branching to cover the maximum volume within the environment. Generally, the filaments (called hyphae) grow outwards through some form of branching. Once this has happened then spore stalks are

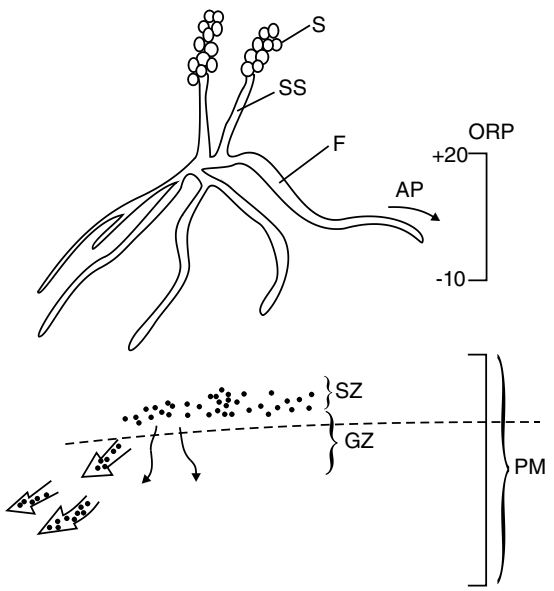

FIGURE 9.12 Diagram of the forms that mold (fungal) growth can be generated in groundwater under semi-saturated oxidative conditions.

developed that become the sites for often massive spore production. It is the spores that are then released into the water to find new environments to colonize.

9.13 PATHOGENIC (HEALTH RISK) PROTOZOA

Protozoa are simple animals that have one or a few cells and, like all other animals, feed on other cells (plant, animal, or microbial) in order to survive. All of the protozoa require oxygen to live and so they are only found in water wells where oxygen is present and there is a food supply (commonly the bacteriologically dominated biomass). Protozoa can feed on other cells commonly by ingesting the cells (which makes even the smaller bacteria more vulnerable). However, there are two major limitations to the ability of protozoa. First they are very inefficient at converting the ingested cells into energy and useful products. Second they do not generally like feeding on cells rich in or coated with, ferric-iron. Commonly protozoa are not considered to be of major importance in water wells. It would be a nice (economic) dream if protozoa could be put into a well and they then digest all of the biomass returning the well to a pristine state. The combination of being very inefficient feeders and neurotic about iron means that there are very few instances where protozoa have been observed biologically regenerating biofouled water wells.

Two protozoa are of potential health risk significance in water wells are *Giardia* (upper figure) and *Cryptosporidium* (lower figure) shown in Figure 9.13.

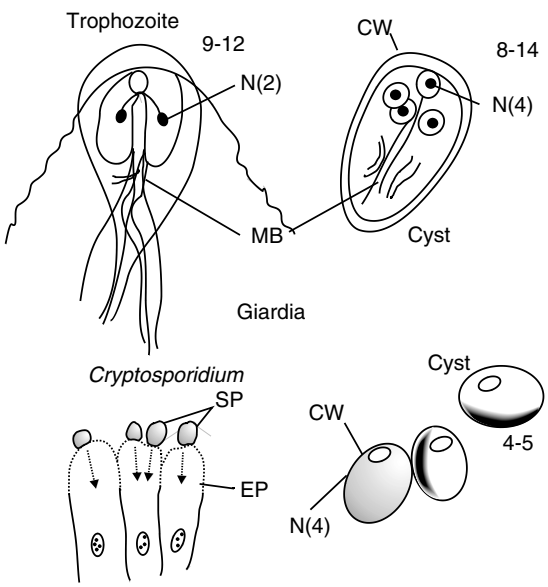

FIGURE 9.13 Illustration of the significant protozoa that can occur in groundwater. The dotted lines show the manner in which sporozoites can enter the epithelial cells in the case of *Cryptosporidium*.

Active cells are shown to the left and the dormant cyst forms to the right. Sizes are shown as a range (in microns). Both protozoa are shown in active states (left) and passive states as cysts (right). There is more than one nucleus (N) with the range shown within brackets. Cyst walls (CW) provide additional protection to these protozoa in the resting phase. *Giardia* contains specialised median bodies (MB) that are very distinctive while *Cryptosporidium* has generating sporozoites (SP) that can then enter the host's epithelial cells (EP).

Both of these protozoa can cause infections in humans consuming potable water contaminated with these cells or spores. In general both species of protozoa can occur in well water that has been under the influence of surface waters but these organisms are not capable of forming a part of the normal biomass that is formed in water wells. Evidence also suggests that the natural filters around the wells will tend to remove and destroy these protozoa. Infections related to *Guardia* or *Cryptosporidium* are therefore most likely to either directly, or secondarily involve a surface water source.

9.14 SIZE OF VIRUSES AND BACTERIA

Evidence to date indicates that it is the bacteria, as a group that dominates water well environments and aquifers. However, much of social anxiety is directed these days at viruses as the perceived causes of all infections. This concern has recently (last decade) got so blurred that many in the communications industry place all microbial infectious agents as being viruses including many bacterial species such as those causing typhoid, diarrhea, and pneumonia. Bacteria are a lot larger than viruses and have the ability to be able to reproduce in a favorable environment. Viruses cannot reproduce themselves but have to parasitize some plant, animal or microbial cell in order to do that. They have to take over much of the functioning of the cell simply to reproduce the virus particle at the expense of the cell. Only one group of viruses (bacteriophage) can actually infect bacteria and then in a selective manner. Most viruses in groundwater are more likely to be passive survivors until they become accumulated within the biomass and degraded.

In Figure 9.14, the upper diagram shows the relative size of bacteria (top) and virus particles (bottom) using the micron scale. Lower diagram shows three typical virus particles in which the upper two are animal while the lower one is a bacterial virus. These particles are not capable of reproduction without a suitable host cell and they are not known to possess defense mechanisms against accumulation and destruction by bacteria consortia in slimes or colloids associated with the biomass. Typical plant and animal virus are shown in the center of the diagram and a typical bacteriophage below. All have some nucleic acid (NA) structures but only the animal and plant viruses have envelopes (EV). These viruses contain capsid materials (CM) and glycoprotein surface protrusions (SP). Bacteriophage appear more complex having a head (HD), stalk (SK) and attachment (AT) that can pull the phage down to a suitable surface.

This bioaccumulation of virus particles into the biomass and subsequent degradation means that the numbers of virus particles that can be recovered from

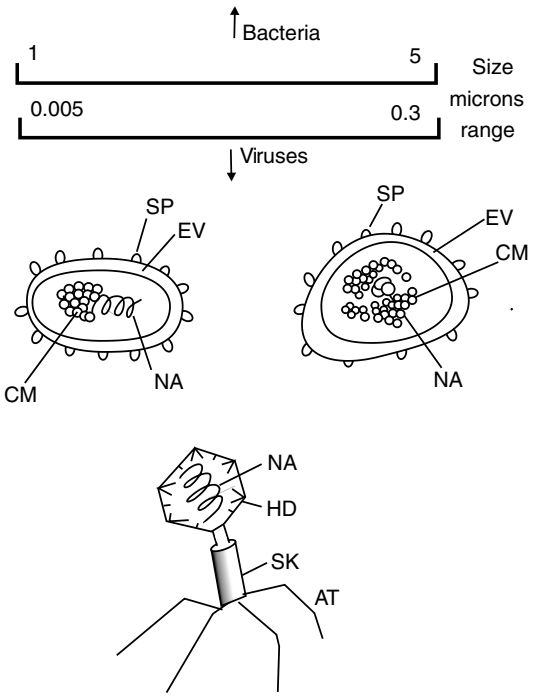

FIGURE 9.14 Relative size and shape of virus particles in comparison to bacterial cells.

well waters is most likely to be low. For virus particles reconsidered to represent a health risk to humans, it is therefore common for liters of water to be filtered before any viruses can be recovered and identified.

9.15 GROWING BACTERIA

A single bacterial cell is far too small to be directly observed and so methods to culture (grow) bacteria have to involve the observation of a mass of bacteria that has been grown. This observation can take a direct form such as noting that the clear liquid has gone cloudy with growth or there has been a growth of cells collectively large enough to be visible (colony). Indirectly the presence of active bacteria can also be determined by their reaction with a given growth medium. Figure 9.15 illustrates these various methods applicable to the determination of presence–absence or populations of active cells in the sample being tested.

Upper left diagram shows a typical liquid culture in which the bacteria grow (G) in the liquid suitable nutrient broth (B) within a culture tube (CT) that is transparent and that allows the growth to be seen as cloudy or gel-like forms through the tube. Upper right is a downward angled view of a Petri dish (PD) contained gelled solid agar medium (AM) on the surface of which distinct colonies (C) of bacteria are

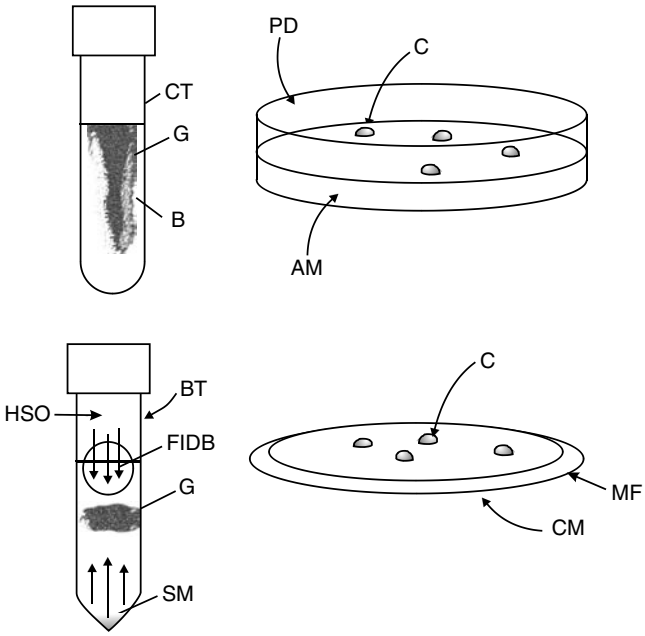

FIGURE 9.15 Diagram presenting the basic cultural techniques in common usage for bacteria.

growing as separate clumps. Lower left is a biological activity reaction test (BART, BT) tester in which there is a floating intercedent device ball (FIDB) commonly referred to as a BART ball that restricts the diffusion of headspace oxygen (HSO) down (arrows pointing down) into the sample under the ball. Selective crystallized chemical media (SM) in the base diffuses up (arrows pointing up) to trigger growth (G) that commonly occurs first at the redox front. Color reactions are often involved that helps with diagnosis. Lower right is the membrane filter (MF) technique through which the WS is filtered. When this is done the microbial cells become entrapped in the 0.45-μm filter. This MF is then placed on a culture medium (CM) and incubated. Bacteria from the MF that are able to grow on CM then produce identifiable and countable colonies (C).

All four of these bacteriological techniques have a different purpose. Using the broth culture method usually allows a presence or absence determination. The use of an agar plate does allow the number of collies to be counted and a population projected as colony forming units (cfu). The BART test is semi-quantitative in that the time lapse to the first growth can be used to predict the population as predicted active cells (p.a.c.). Membrane filtration can generate a quantitative estimate on the basis of the number of colonies that were recognized on the filter.

9.16 AGAR DIP-PADDLE TECHNIQUE

One technique that has been widely used is the use of the agar dip-paddle. Here rather than streak the sample onto agar in a Petri dish, the agar is immersed in the WS to be tested. Bacteria from the sample are attracted to the nutrients in the agar and some of them will attach to the agar surface. This takes a specified length for immersion (see manufacturer's recommendations) after which the agar dip-paddle is removed and returned to its container for incubation and the possible growth of bacterial colonies. These devices are very convenient to use but they tend to have a short shelf life since the agar on the paddle is commonly very thin.

Three stages are shown in the use of the dip-paddle technique. In Figure 9.16, upper left shows the plastic dip-paddle (DP) holding the agar medium (AM) and supported (S) by a plastic tray-like structure from the cap. Middle right diagram shows the method for testing the water using a dip-paddle. Here the dip-paddle is lowered (arrow down) down into the sample (S) for a set time (manufacturer recommended) into the water. This exposure time (ET) is critical to ensure that bacteria do attach (BA) to the AM. The dip-paddle is then removed (up arrow) and returned to its container for incubation. After a set incubation (I) times then colonies that have grown on the agar are counted (CC).

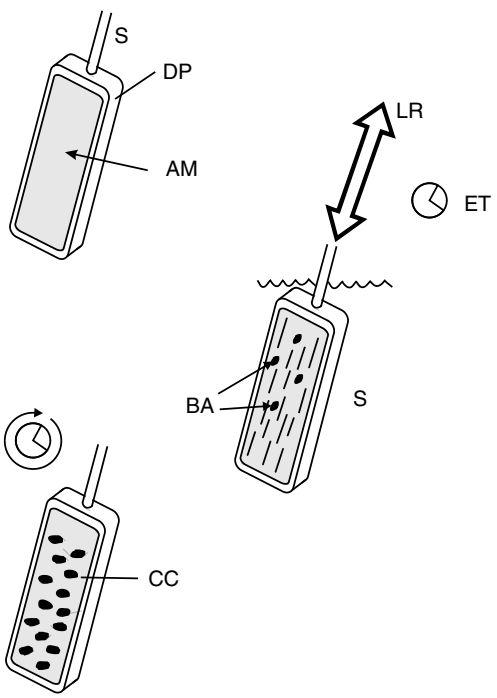

FIGURE 9.16 Schematic of the agar dip-paddle technique.

9.17 SLOPE BART CULTURE TECHNIQUE

As an alternative approach to the dip-paddle, the slope BART tester (SB) offers a number of potential advantages since the agar slope (AS) is much thicker and applied within an inner vial (IV) that is protected by an outer vial (OV). This patented method involves the addition of the WS for examination to the OV. When added and sealed then the device is turned upside down causing the WS to flow into the IV through the window (W). Turning the device back upright causes the water inside the IV to flood the surface of the agar. Reversing the tipping action removes the surplus water from the IV but leaves the agar surfaces populated with any bacteria that were in the sample and attached to the surface. When incubated, these bacteria (that can grow on the agar) will grow to form distinctive colonies can be counted (CC). This is just like in the agar spreadplate and the dip-paddle methods. The advantage of the BART-SLOPE technique is that the shelf life and the incubation times are considerably longer allowing their use for prolonged incubations under very dry conditions.

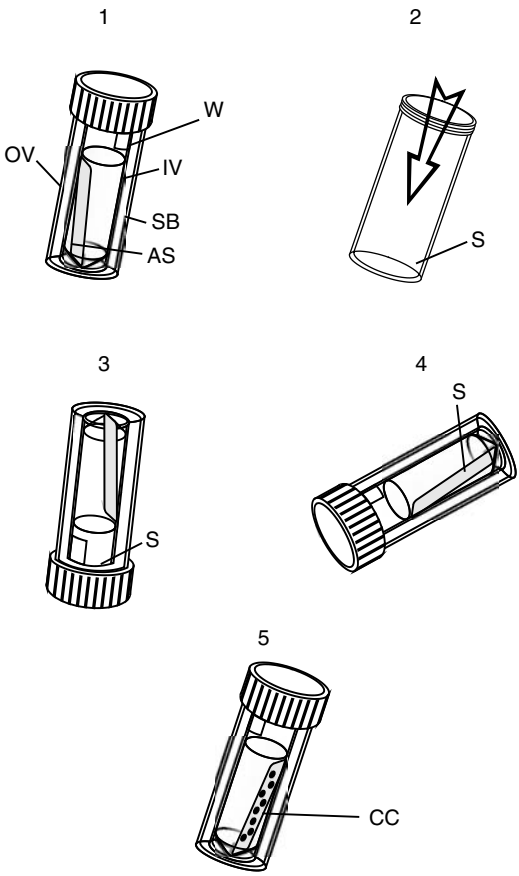

FIGURE 9.17 Methodology for the utilization of the slope BART tester.

In Figure 9.17, there is an AS within an IV protected by an OV. A window (W) is cut in the upper part of the IV to allow any WS placed in the OV to move into the IV and flood the agar in the SB. To conduct the test the sample (S) is first added (10 mL) to the OV separately and then the SB is reassembled. The device is now turned upside down on its cap so that the sample S now enters the IV through W. Tilting the SB back causes some of the WS to flood over the AS. Reversing the process removes the surplus sample from the IV which is then incubated. Colonies can be counted (CC) once there has been an adequate incubation time for the bacteria to grow. Because there is so little water loss from this test method compared to the agar spreadplate and the dip-paddle techniques then it is possible to prolong incubations for weeks or even months if necessary.

9.18 BART TEST METHOD

Biological activity reaction testers (BART™, Droycon Bioconcepts, Inc., Regina, Canada) were patented by Cullimore and Alford on the premise that the water sample being tested would be presented with a much greater range of environments than could be achieved than using an agar plate or broth. This was made possible by crystallizing the selective nutrient into a pellet form on the floor of the test vial (so that the nutrients would diffuse upwards once water was added) and a floating ball placed at the top of the water column in the vial to control oxygen moving downwards into the water column from the headspace. Fifteen milliliters were found to be the optimized volume that would maximize the variety of dynamic environments that were created in the test vial from RD (oxygen-deficient) at the bottom of the vial to OX (oxygen present) just below the ball. This strategy allowed the bacteria of interest time to find a suitable environment and then become active. From this it was found that the time lapse to the first observation of activity could be linked to the population size in the WS. In simple terms, the bigger the active population then the shorter would be the time lapse.

In the dried-sealed form, the BART tester has a shelf life of 4 years. It consists of an OV, and IV containing a FID Bart ball (FIDB) and selective medium (SM) as a dried pellet. To charge the test with a liquid sample, then it is necessary to remove the outer cap and take out the IV. Pour 15 mL of the sample to be tested into the IV until the FIDB floats up to the fill line (FL). Recap the IV and place back in the OV and screw down the cap. Incubate the BART tester and observe for reactions (R) on a regular basis (daily for semi-quantitative and more frequently to achieve better precision). Note the time lag to the first reaction can be used to compute the population using BART QuickPop software version 3.1. Incubation temperatures recognized are 10°C–12°C, 21°C–23°C, 26°C-28°C, and 35°C–37°C with room temperature being the most commonly employed. Laboratory BART testers do not use the OV components for the test and should be used only in the laboratory setting by technologists familiar with normal microbiological laboratory procedures. Reactions are listed for each type of BART tester and some test can be performed using the sorption (HAB-BART) system or direct video (V-BART-READ) system in which the temperature can be controlled automatically (Figure 9.18).

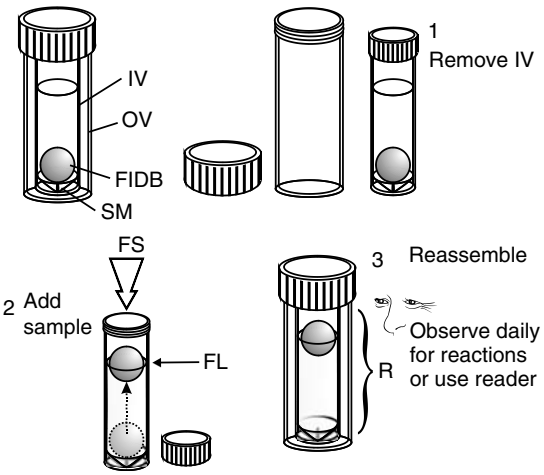

FIGURE 9.18 Standard methodologies for the application of the BART tester.

9.19 RODINA TEST FOR IRB

In the late nineteenth century, there was a real concern about waters that were iron rich. These waters mostly came from wells and often experienced secondary problems that we know now relate to various biofouling events. With the advent of the golden age of microbiology (essentially 1875–1915), there was recognition of the potential importance of bacteria and in particular the iron bacteria. David Ellis in 1919 published a work on "iron bacteria" which remains a solid text even today well worth reading. Because it was found that various forms of iron bacteria were commonly found in well waters, there were a number of attempts to develop a quick and easy test that would allow the well users to determine the nature of the problem in the well. At that time there were neither video camera that could examine the inside surfaces of the wells nor was there a suite of scientific test that could be applied to determine the nature of the chemistry and biology of those wells. One thing common with the tests in Figure 9.19 through Figure 9.22 is that they were all very simple and yet at the same time very effective for common use.

This test method was developed by Rodina. Here a wide-necked flask (WNF) is used into which the sample (S) is added to a depth (D) of approximately 3 in. (10 cm). Rodina found that this depth not only allowed sufficient oxygen penetration for the aerobic (ferric-iron producing) bacteria, but also deep enough to allow anaerobic (oxygen free) zones to develop on the floor of the flask where the iron-reducing bacteria would be active. Rodina found that when the WNF had been charged and then left overnight, it was possible to observe growths. Two forms of growth became recognized. These were a light flocculant (LF) material resembling discolored cotton wool; and a denser granular (DG) material which, generally, settles to the bottom of the WNF.

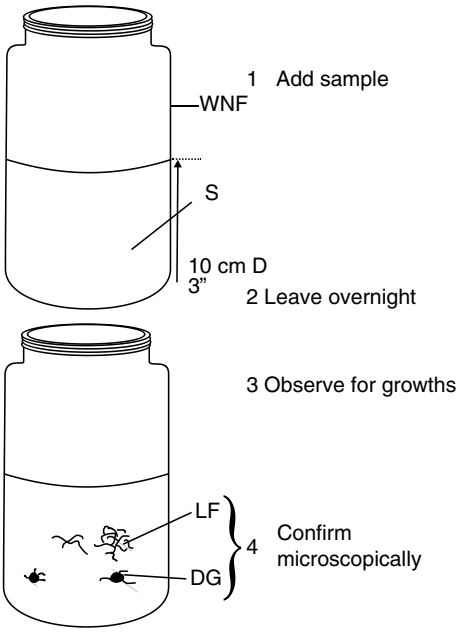

FIGURE 9.19 Simple test methods for iron-related bacteria in water samples, Rodina test.

The method was very simple and did not involve adding any nutrients or chemistry to the test. The overnight generation of growth was therefore a signal that there was a very considerable level of microbial activity in the WS. It would not be so sensitive to iron bacteria in water that were less active.

9.20 CHOLODNY TEST FOR IRB

One of the shortcomings of the Rodina test method was the lack of surfaces (other than the water surface and walls of the flask) onto which iron bacteria could attach. It was obvious to many of the early observers of iron-bacterial growths was that they commonly attached to surfaces and would even grow out into the water flow as an iron-rich slime-like mass. Cholodny saw the need to increase the surface areas onto which the iron bacteria could attach. This, he achieved by floating a microscope slide under a cork in the sample being tested. The advantage to this was that not only was there a vertical surfaces surrounded by the WS but also that the slide could be removed and examined microscopically for the types of growth. This also allowed the cells to be stained so that a better understanding of the nature of these iron bacteria could be obtained.

This Cholodny method employs a wide-necked glass jar (GJ) into which the sample (S) is added with the water level (WL) set high enough to allow the device to float freely in the water. This device uses a cork (C) to maintain buoyancy for the microscopic glass slide (GS) which had been placed firmly into the cork. This technique involved daily observations with the objective being to observe the presence of either dense granular specks (DS) or surface light granular (SLG) material resembling

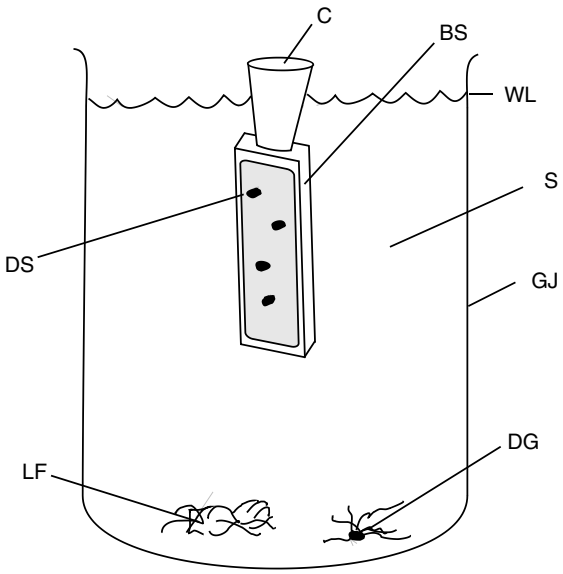

FIGURE 9.20 Simple test methods for iron-related bacteria in water samples, Cholodny test.

discolored cotton wool (PSS); or dense granular (DG) on the plastic stir stick. This can also occur on the soft steel washer (SSW) on the floor of the conical flask (CF).

The advantage for the Cholodny method was that it now became possible to monitor the activity as it generated which gave an idea, through the time lapse measured in days, as to how active the iron bacteria were in the sample. Staining of the slide contents after removal from the WS now allowed an identification of the principal iron bacteria that have distinctive cells shapes (such as *Gallionella*, *Sphaerotilus*, and *Leptothrix*) and also examine any slime growths that had attached to the slide.

9.21 GRAINGE AND LUND TEST FOR IRB

Grainge and Lund recognized in 1969 the shortcomings of the various proposed techniques for the simple culture of IRB and modified the technique to stimulate the growth of these bacteria. The floating glass slide used by Cholodny was replaced by a white plastic coffee stir stick that was sloped into the sample (S) under test. This use of a stir stick was found to provide a much more stable platform for the iron bacteria to grow on in a recognizable way. This was partly because the white background on the stick provided a much better back drop for observing hanging and flocculant growths when these occurred commonly just below the surface of the sample. Additionally, a soft steel washer (SSW) was dropped into the flask. This washer would sink to the floor and then provided a ready source of iron with some nutrients (sulfur and phosphorus) and active electrical charges. The washer now became a very attractive site for the growth of both iron-oxidizing and iron-reducing bacteria which can then be easily recognized.

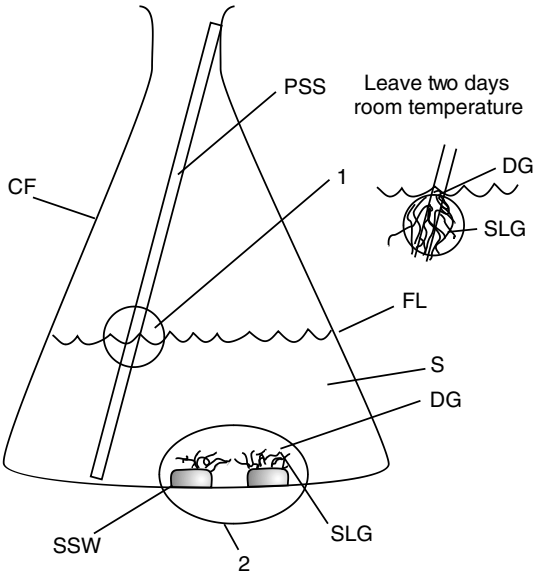

FIGURE 9.21 Simple test methods for iron-related bacteria in water samples, Grainge and Lund test.

This method uses a 250 mL conical laboratory flask. To undertake the test then the water sample (S) is added to about one-third of the fill volume (e.g., 100 mL) for the flask (FL). A soft mild steel 1 in. (uncoated) washer (SSW) is placed into the WS after dispensing. This washer should sink to the bottom and may entrap a few bubbles. Do not try to dislodge the bubbles and simply add the white plastic stir stick (PSS). This stir stick should be at least 6 in. long. The test is left for 2 days at room temperature and observed for the presence of IRB growth at the interface (1) between the PSS and the fill line (FL) surface where DG and string like growths (SLG) would be taken to be positive indications of IRB activity. On the uncoated mild steel washer (SSW) then the presence of dense granular growths emanating from the surface of the washer may also be considered to be a positive.

9.22 GEORGE ALFORD QUICK AND DIRTY (GAQD) TEST FOR IRB

Creativity sometimes comes from a mixture of frustration and desperation with a client wanting an answer the next morning. Such is a condition that can drive one to seek solace in saloon bars along with a Jack Daniels and a few friends to engage in the same quest for an answer. For George Alford who would occasionally seek refuge in such a place and on one occasion, the glint in the glass of whisky gave him the answer for a quick and dirty test for IRB. The reasoning went like this: (1) Jack Daniels contains alcohol and perhaps these iron bacteria in the sample could feed on that organic compound when diluted to more modest levels; (2) the top of the refrigerator

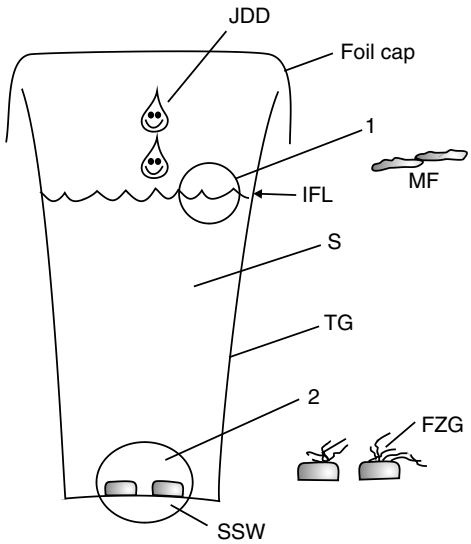

FIGURE 9.22 Simple test methods for iron-related bacteria in water samples, George Alford test.

in the corner of the saloon is perhaps warm enough to allow the iron bacteria to grow quickly; (3) to hell with a conical glass flask that is expensive, let us use a regular 12 oz glass; (4) heck let us put that uncoated soft steel washers into the water, fill the glass with the sample and then why not add a couple of drops of Jack Daniels to feed the bugs (really one drop was for the test and one was for the tester!); (5) well we do not want the water to evaporate and so lets put an folded aluminum foil cap over the glass; now finally (6) let us place the tumbler on the warm top if the refrigerator and leave it overnight. The next morning the iron bacteria had feasted on the Jack Daniels, grown around the steel washer and the quick and dirt test was created!

This GAQD method uses a tall glass (TG) commonly found in bars and restaurants. Sample (S) is added so that the imaginary fill line that is three quarters of the way up the TG. A soft steel uncoated washer (SSW) is added to the test apparatus and then essential two drops of Jack Daniels is added as the primary nutrient source for the IRB (they like their scotch too!). The test is left to stand overnight generally on the top of a refrigerator (where it is warm) and examined. Metallic floaters (MF) on the surface of the WS and fuzzy growth (FZG) on the washer are both considered indicative of a positive for IRB. This test has found application particularly in the southern States of the Union where even IRB enjoy their whiskey!

9.23 STAINING METHODS FOR IRB

From the early part of the nineteenth century until mid way through the twentieth century, there was a preoccupation with finding ways to stain cells followed by the use of differential chemicals that could identify even strain of bacteria. Identification

of the IRB by staining is made in some cases easier since the (commonly ferric) iron does given colors to the cell masses whether in the form of bioaccumulations around the cell or inside the cell. This easy identification of at least some of the IRB led to the development of microscopic procedures that are still in use to day and do not require staining. For example, *Gallionella*, *Sphaerotilus*, and *Leptothrix* can be separated by one having a ribbon-like tail (*Gallionella*), while the other two have distinctive tube-like sheaths within which the bacterial cells commonly reside.

Three staining methods are shown in Figure 9.23. Olanczuk-Neyman and Meyers methods are shown at the top, Leuschow and Mackenthum method in the middle and the standard negative stain is shown at the bottom. The upper two methods both use a membrane filter soaked in oil (MFO) to achieve good transparency for the recognition of the iron-related bacterial cells. In the upper diagram bacterial cells (BC) stain red while iron deposits stain blue. In the middle diagram both *Gallionella* filament (GF) and sheathed bacteria (SB) can be seen. For the negative stain (below) there is a blackened nigrosin backgrounds (NB) with the bacterial cells (BC) clearly recognizable by their shapes and capsules (C) can also be observed as transparent zones outside of the cells.

While these staining methods can generate clarity for the identification of the sheathed and ribbon bacteria they do not differentiate the bacteria that occur within gelatinous biomass. These "clumps of gel-like material" are commonly ignored in the microscopic examination of the sample even though such particles may contain a greater number of viable IRB and actually be the dominant cause of the problems associated with the water well.

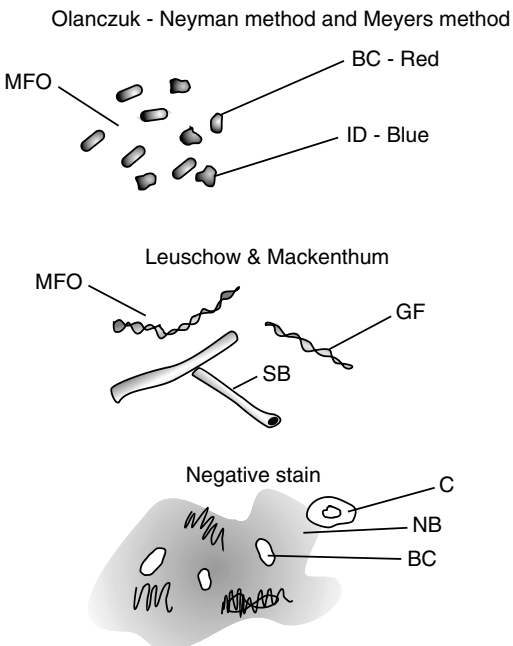

FIGURE 9.23 Illustration of the various staining methods to identify iron-related bacteria.

9.24 AGAR SPREADPLATE ENUMERATION METHOD FOR BACTERIA

From the early part of the nineteenth century, it was recognized that bacteria could produce growths on surfaces and from the 1850s to the 1940s it was common for bacteria to be grown on the "sterile" surfaces of sliced potatoes. Attempts were made to use gelatin as a surface for growing bacteria but very often the bacteria would digest that surface leaving a stinky mess. In the 1880s, a new surface was used based upon agar–agar (an extract from seaweeds used to make jellies). The bacteria did not normally break down the agar and often grew to form distinctive clumps of growth that became known as colonies. Today agar is widely used at concentrations of between 1.2 and 1.8% in the culture and identification of bacterial colonies respectively. Today this is known as the agar spreadplate method and is commonly used to enumerate bacterial population as colony forming units (cfu).

Figure 9.24 is set in three parts with the top section showing preparation of the sample for an agar spreadplate, the middle part showing the act of streaking the sample over the agar while the lower part illustrates the methods used in a pour plate. Upper section shows the approach to three common types of sample. Solid samples are dispersed physically prior to dilution, semi-solid samples may disperse when added to water or may require physical dispersion prior to dilution. Some liquid samples are diluted with the objective getting acceptable numbers of colony growth to be enumerated (usually between 30 and 300 colonies). Streaking the diluted sample over the selective algae medium (SAM) is shown using a sterile metal alloy loop (L, in the middle diagram). In a pour plate, the diluted sample (DS) is placed into a Petri dish and molten agar medium is poured over the sample.

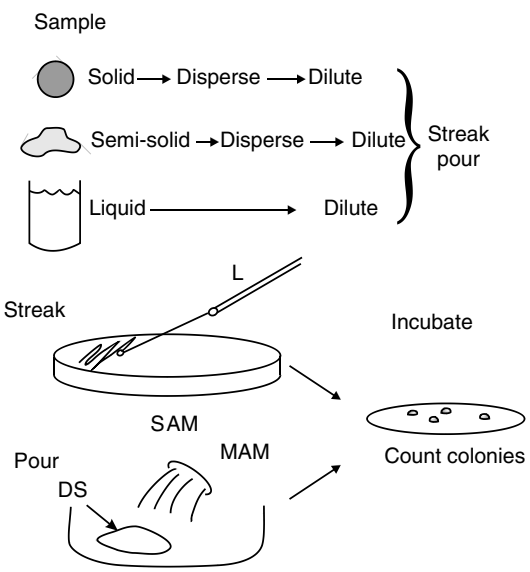

FIGURE 9.24 Agar spreadplate enumeration of bacterial populations.

The temperature should be just above the setting point for the molten agar medium (MAM) that commonly sets at 46°C–48°C so that the agar sets as disperses the sample. After incubation then the colonies are counted. In all cases the populations are recorded as cfu.

9.25 BACTERIAL COLONY FORMS GROWING ON AGAR PLATES

From the very beginning of bacterial classification, emphasis was placed on the manner in which bacteria grow on surfaces. These surfaces included potato slices, gelatin discs, and then agar plates after the inventions of the Petri dish and the refinement of selective agar culture media. Bacterial growth occurs over these surfaces from small clusters of cells that multiply and become visible at around 0.2–0.4 mm. These bacterial colonies come in distinctive colors and sizes; have different profiles, textures, and manners in which the growths move out over the agar surface. Even the smells can be distinguishing (even if sometimes unpleasant!). Color is one of the most distinguishing features but generation of the color is sometimes affected by the presence of light and also the age of the colony. Generally older colonies tend have more distinctive colors (pigments).

Figure 9.25 shows only some of the typical structures that are seen in bacteria colonies. Classical domed (D) colonies resemble hills of cells growing out of the agar and they commonly have a simple circular shape with the profile domed to some extent. Other colonies spread out evenly over the agar surface to form a flat (F) form of growth. Sometimes these types of flat colonies can spread (SP) out over the agar surface in an erratic manner or they can virtually cover the whole of the agar surface. This is called swarming. Some bacteria only generate small pin point (PP)

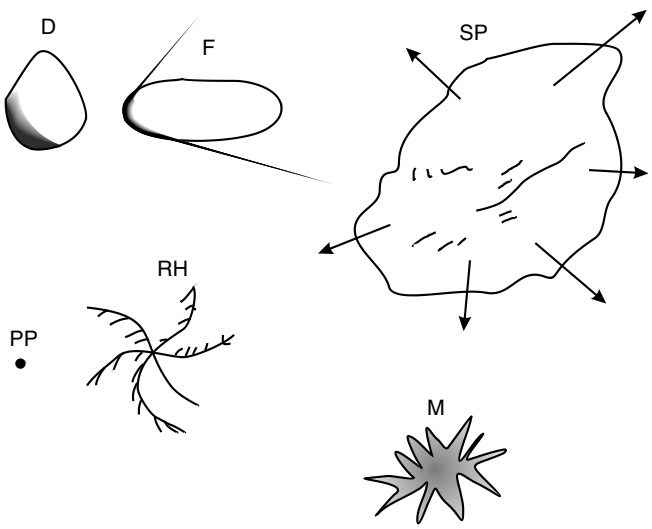

FIGURE 9.25 Illustration of the major types of colonies that can grow on agar surfaces.

colonies that can sometimes only be seen with a low power microscope. Other bacteria produce whirling forms of growth called rhizoids (RH) while fungi commonly produce mat-like (M) growths that generally look woolly in form.

9.26 ATP TEST METHOD FOR DETECTING BACTERIAL ACTIVITY

All living cells possess a common characteristic that relates to the storage of energy. Like many machines, cells also have a means of storing and releasing energy in an intelligent manner to meet the demands created by the cells in its environment. Perhaps surprisingly all cell whether animal, plant, or microbial posses the same biochemical method for high energy storage. This method involves ATP. It generates high energy phosphate bonds that are released to meet the cells demands for energy. Traditionally, ATP has been measured because its energy can be released as light when the enzyme luciferase as applied. Here the amount of light (as relative light or luciferase units, RLU) released is equivalent to the amount of ATP in the cell. The more light emitted, the more ATP was present and the greater the amount of cellular activity occurring within the sample being tested. Luciferase is commonly found in fireflies and the bioluminescent bacteria. Those glowing spots on fish in deep ocean environments are all a result of activities of these

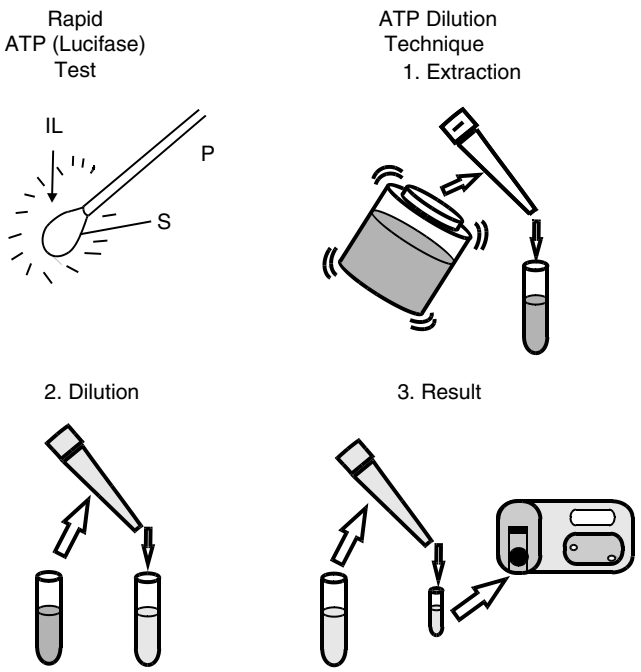

FIGURE 9.26 Diagram of presence–absence adenosine triphosphate method for the rapid detection of active microorganisms in, or on, a sample.

bioluminescent bacteria burning up ATP to make light! When it is necessary to estimate the amount of microbial activity in water, soil, food sample then the estimation of the ATP content can generally be used to predict the population size for the active cells in the sample. Adenosine triphosphate does not however differentiate the types of microorganism (or any other cells for that matter) that might be active. It remains however a very good first line of defense to determine whether active cells are present in the sample. Beyond that then the BART testers can be used to differentiate the microbial communities involved.

Two methods are shown in Figure 9.26 for the application of the ATP technique for the detection of active bacteria. The upper diagram is a rapid (luciferase) test that uses a swab (S) containing the reagents for the ATP test. When the swab is moved over a material that is rich in active bacteria then light is emitted from the swab. Therefore, this is a presence–absence technique. More comprehensive test methods use a luminometer to measure the amount of light being emitted as the ATP is broken down by the luciferase enzyme system. Manufacturers do have standard conversion formulae for converting the RLU into ATP as an index of the number of active cells that were detected by this method.

9.27 COMPARISON OF ATP TO TIME LAPSE USING BART TESTERS

Both the ATP and the BART testers determine the level of microbial activity in samples. Adenosine triphosphate is broad spectrum and is based upon the activity of all cells involve in high-energy phosphate bonds. The BART testers are more specific to determining the cultural activity of selected communities of bacteria, such as IRB or HAB. Primarily, most BART testers employ a time lapse as the means to estimate the levels of activity in the community at the time of testing. The two analytical approaches (ATP and time lapse) both determine activity levels in the sample under test as RLU concentration or time lapses commonly measured in seconds, hours, or days. There is no selectivity in the ATP test and the measurement is of all sources of active ATP although there are techniques to separate out simple cells (microbiological) from complex tissue cells (plants and animals). Selectivity in the BART tester is determined by the type of BART being employed. For example, communities of IRB are best determined using the IRB-BART tester. In Figure 9.27, there was a semi-quantitative relationship between the log of the time lapse observed in seconds (x) when compared to the log of the RLU (y). These links were semi-quantitative with a regression correlation (R^2) was 0.79.

$$\text{Log RLU} = [x(1E + 0.8x - 1.65)]$$

This comparison used water and wasteWSs and the time lapses were determined using the HAB-BART system which generated a digital time lapse at the time when one of the sorption values fell by $>20\%$ indicating that the methylene blue was moving from an OX (blue) to a reduction (clear) state. While clearly more research is ongoing to obtain improved correlations, it is reasonable to draw comparisons

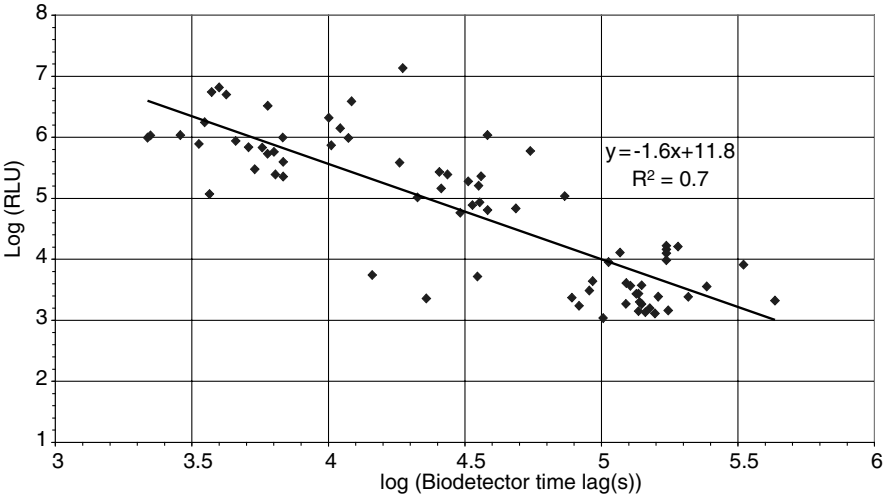

FIGURE 9.27 Comparison of log time lapse using the HAB-BART system and the log of the RLU analysis as a direct indicator of the adenosine triphosphate activity level in the water samples.

between the HAB-BART system and the ATP detection methods as two different methods of measuring activity.

Figure 9.27 shows a log–log comparison of the time lapse (in seconds) generated by the HAB-BART system and log RLU as a direct indicator of the ATP activity levels in the sample.

9.28 THEORETICAL FUNCTIONING OF BART TESTER

Biological activity reaction test (BART™, Droycon Bioconcepts, Inc., Regina, Canada) is a patented method for selective determination of the activity levels of selected bacterial populations in a given WS. Activity is defined as a combination of all events that are associated with growth (increasing biomass) or metabolism (sustaining the living cells). BART testers apply a combination of selective chemicals diffusing up from a crystallized pellet in the floor of the tester; and oxygen diffusing down around the floating BART ball. When the water samples to be tested is added to the tester then there are sequenced events including interaction between the chemistries of the sample and the diffusion fronts forming as the selective chemicals diffuse upwards and the oxygen from the headspace diffuses downwards.

Two diagrams are included in Figure 9.28 and these are set side-by-side. The left-hand graphic shows the components employed in the generic BART tester while the right-hand shows the functionality by which selected activities can occur. This diagram therefore shows the tester in its double-walled (IV inside the OV) version that is commonly used for remote testing of samples away from a laboratory setting. This is known as the field version. Test can be performed more economically using

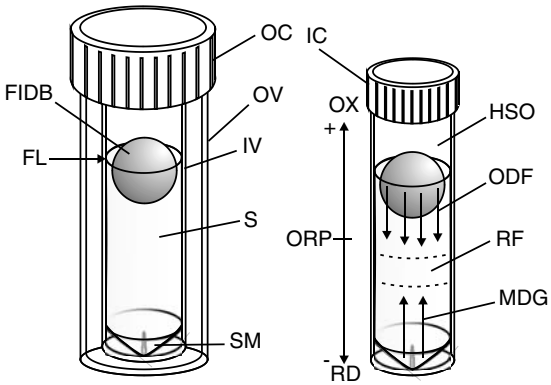

FIGURE 9.28 Schematic of the basic functioning of the BART tester.

just the capped IV and this is more suited to laboratory use and hence is named the "lab" version of the BART tester. BART testers are always supplied in sealed aluminum foil packing which extends the shelf life to 4 years.

Functionality of the BART tester is the focus of the right diagram in Figure 9.28. The vertical scale to the left of the figure shows the ORP running from OX above to RD beneath. These values are expressed in millivolts (ORP). Oxygen comes down around the BART ball from the HSO in the air to create and an oxygen diffusion front (ODF). At the same time a medium diffusion gradient (NDG) is moving upwards through a transitory redox front (RF). Time lapse is that period of time between the start of the BART test and first observation of a recognizable reaction and this is used to determine the activity level in that selected group of bacteria within the BART tester.

9.29 TYPES OF BART TESTERS IN COMMON USE

There are seven major types of BART tester in routine use in groundwater applications. Each is differentiated by the selective crystallized medium that is employed and they are recognized by the colors used for the caps. The labeling is also specific to the individual type of BART tester and relates to the activity (aggressivity) at the semi-quantitative level based upon the time lapse (days). Happy and sad faces are used to differentiate the primary difference between background and challenging levels of activity in the sample. Happy faces denote acceptable results with very low activity in the sample for the specific group of bacteria under test.

Figure 9.29 consists of a chart showing the seven most commonly used BART types in groundwater testing. Each BART type is delineated by the color of the cap (described), and the type of bacteria detected using that system. These colors are related to some extent to the type of bacteria being examined for. For example, the blue-capped HAB-BART indicates that the principal characteristic being detected is the presence of oxidized methylene blue which has a blue color. The IRB-BART has a bright red cap indicating the relationship to ferric-iron that can have colors

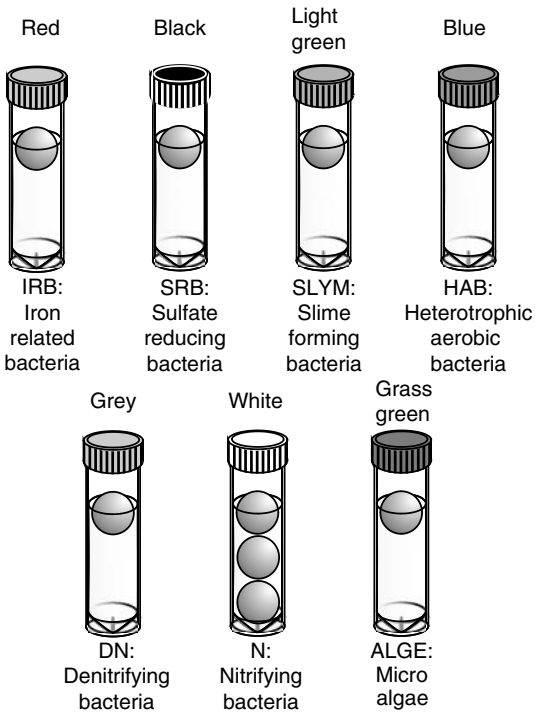

FIGURE 9.29 Different cap colors for the various types of BART testers.

ranging through orange to red and brown. Black sulfides are the product of the presence of SRB in the SRB-BART and so here the cap is black. Slime-forming bacteria are very common and commonly grow more quickly than any other bacterial group and so a light green has been used to designate the SLYM-BART tester. Grey wastewater that has been exposed to oxygen would tend to generate nitrates which are formed by the nitrifying bacteria (N-BART tester) using a white cap, while nitrates that are reduced by the denitrifying bacteria (DN-BART) employ the grey cap. In the suite of BART testers it is the test for micro-algae that is very different because of the methodology which is very different. This tester is laid upon its side and illuminated in order to encourage the growth of the micro-algae that does include many members of the Cyanobacteria (blue-green algae). The cap for this test is dark (grass) green.

9.30 PROTOCOL FOR FILLING BART FIELD TESTER

Figure 9.30 shows the filling of a BART tester in six stages (numbers 1–6). The first stage (1) shows the fully assembled field tester ready for starting the test. This tester has been removed from its outer sealed aluminum foil pouch and placed on a clean bench top. The contents of the BART are sterile and have been subjected to the DBI standard ISO quality management procedure before being sealed in the foil

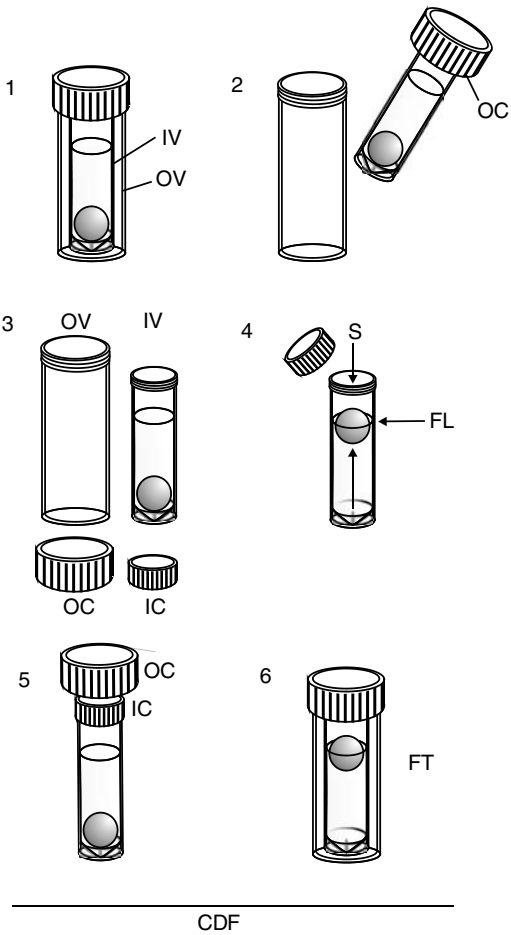

FIGURE 9.30 Standard protocol for setting up most BART field tester.

pouch. Both the outer and the IVs are sterile and they should be handled following the directions listed below. First remove the tester from the foil pouch. The next step (2) involves the outer cap (OC) being unscrewed and placed down on a clean surface without turning it over. The IV is designed to lift up with the outer caps removal and so the IV remains suspended below the inner cap (IC) inside the outer cap. The IV should be carefully taken out of the outer cap and placed (cap side up). For convenience, the OV and IV can be rested on their caps that are placed screw sides downwards. The next stage (3) now illustrates the IV being uncapped and prepared for the sample (S) to be added. The WS should be added until the water has reached the fill line (FL) at which time 15 ± 0.2 mL would have been added to the IV. It should be noted that as the sample is added (4) the BART ball (BB) floats up to the fill line. Once in this position the floating BART ball now restricts the movement of oxygen from the headspace into the WS and so there is much less oxygen moving downwards into the water column. Once sample has been added then the capping of the tester (5) can

be undertaken by first screwing down the IC onto the IV, placing the capped IV into the OV and then screwing down the outer cap. The IV will automatically seat itself inside the OV when this is done. It should be noted that neither cap should be tightly screwed down. This is because overtightening can compromise the plastic and cause fracturing of the plastic to the detriment of the test. The last stage (6) shows the manner in which the field tester (FT) is placed for incubation on a clean dry surface away from direct sunlight. It should also be noted that the tester once charged should normally not be shaken since this would lead to a DR of the diffusion fronts that would be forming in the tester.

9.31 COMMON SITES FOR EARLY BART REACTIONS

Each BART tester tends to have different sites where the first reaction occurs. This is critical to the determination of the time lapse and allow the activity and population size to be determined. Figure 9.31 is an illustration of the IV containing the water sample (S) under test. This sample would be 15 ± 0.2 mL in volume but there would be a number of places within that volume where the first reaction (and subsequent reactions) may occur. These are shown as a series of lateral zones (or regions) indicating where reactions and activities can occur within a generic tester. Each region is numbered in a descending order.

Uppermost (1) is the region where the water surrounds the BART ball (BB). Here the critical factors are the high surface areas created by the curved ball and the

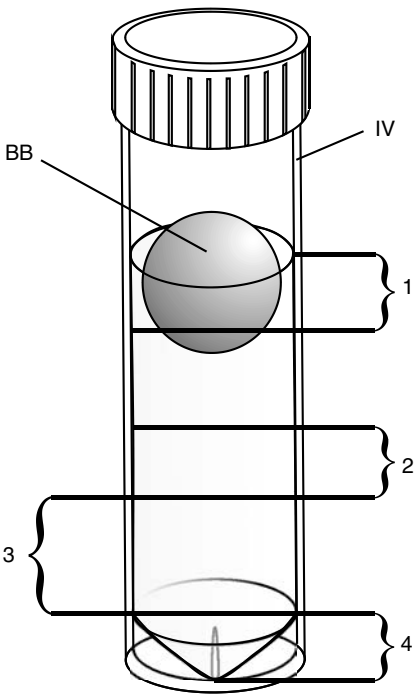

FIGURE 9.31 Illustration of the common sites for first reactions in BART testers.

flat sides of the IV, relatively small water volume, and an abundance of oxygen dissolving into the water from the headspace atmosphere above. It should be noted that >95% of the oxygen comes from this source and <5% permeates through the plastic walls. Dominant reactions that occur here are the generation of foam (FO) rings around the ball due to gas products from fermentation. For a FO ring to be recognized, there should minimally be a connected ring of gas bubbles (GBs) completely around the ball. In the DN-BART such a ring can grow to three or four bubbles in thickness. Another reaction that can sometimes occur is the growth of a biomass around the ball that forms into a slime ring (SR). Just below the BART ball is the location of the redox front (2) which normally forms a common focal site for early clouded types of growth and/or the production of thread-like growths. This zone promotes a considerable number of the early reactions since this is the region where there is a direct interaction between the oxygen moving downwards and the selective chemicals moving upwards.

Further down above the basal cone is a more reductive nutrient rich region (3) where gel-like forms of growth tends to occur more commonly as secondary or tertiary reactions. In the basal cone (4) conditions tend to be very RD and very nutrient rich. Here the types of reactions are more from the daughter products of activity rather than from the growth of biomass. Chemical reactions to black sulfides and precipitates as white carbonates are two such reactions in the SRB- and IRB-BART, respectively. In the SRB-BART, the black reaction is known as a black base reaction and has forensic value, while for the IRB-BART the white base of carbonates is very common and has more value in the selection of treatments for biofouled water wells than for the identification of the IRB.

9.32 IRB-BART REACTION PATTERNS

Figure 9.32 shows eight of the major reaction patterns activities for the IRB-BART when incubated at room temperature. Reactions are set in two columns with four rows. Each reaction is defined by the two letter standard code and briefly described below:

FO refers to the formation of GBs into a FO ring around the BART ball and discounts any GBs observed forming on the walls or lower side of the tester.

Brown ring (BR) refers to a slime growth around the BART ball that may be glossy or mat in form and is commonly a shade of brown.

BG forms within the bottom third to half of the tester culturing medium and has a jelly-like consistency which will retain shape even when tester is tilted, color ranges from dark red to shades of brown.

BC is a brown-clouded reaction usually middle to dark brown in color.

Green-clouded (GC) reaction that usually starts as a light green moving to a dark green with the clouding starting at the mid-green stage.

Red-clouded (RC) reaction in which the color moves through shades of red terminating in a dark scarlet.

CL is a clouding that forms commonly in association with the redox front half way up the tester. This reaction commonly does not initially involve any brown pigmentation.

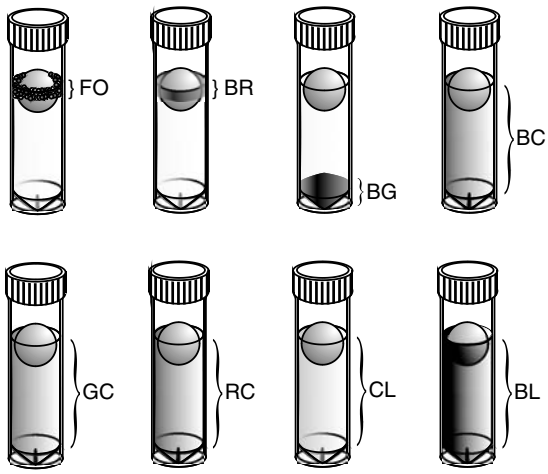

FIGURE 9.32 Standard reaction pattern locations for the IRB-BART tester.

BL is a terminal reaction where the walls of the tester becomes coated (commonly from the bottom up) with black deposits in the basal cone of the tester usually starting near the peg in the middle and radiating outwards.

Negative reactions include the generation of a crystal clear yellow color or the generation of a mid to dark green color that diffuses up from the basal cone. These are both negative reactions unless one of the eight reaction activities listed above is observed.

White base reaction means carbonates have been formed within the basal cone (usually in less than a day) and this indicates that well regeneration should consider the use of an acid to remove these carbonates that may be associated with the biofouling.

9.33 SRB-BART REACTION PATTERNS

The SRB-BART is one of the simplest tests to perform because there are only two primary reactions that are both associated with the generation of hydrogen sulfide and the deposition of jet black iron sulfides at the sites where the bacteria are active. These bacteria are commonly associated with corrosion problems, the generation of black slimes, "rotten" egg odors and taste and odor problems in the water. Wells that have been shut off for a period of time (e.g., several months) are more likely to have SRB-influenced problems.

Figure 9.33 shows the two primary reactions for the SRB-BART tester that are illustrated with two rows of IV testers. In the upper row is shown the BT (black top) reaction in which black sulfide granules from on the lower side of the BART ball and gradually spread out over the surface until the lower half of the ball is black. This reaction is common when the SRB are growing in association with aerobic bacterial activity. These SRB are vulnerable to treatments and can be controlled if effective treatments are applied.

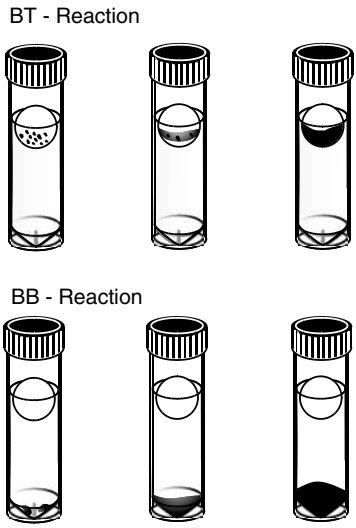

FIGURE 9.33 Standard reaction pattern locations for the SRB-BART tester.

In the lower row is shown the BB (black bottom) reaction which occurs when the SRB are growing under very reductive conditions (anaerobically) within the basal cone of the tester. Here the reaction starts on the sides of the cone and extends until the cones has turned black and the blackening has extended 2–4 mm up the walls of the tester above the cone. Commonly either the BT or the BB reaction will occur first and is followed by the other reaction. If the SRB-BART is read everyday then on some occasions a blackening may have occurred both in the base and around the ball. This is called a black all (BA) reaction and in practice this most commonly occurs when a BB reaction is dominant over a BT.

Clouding in the culturing medium is not significant in the test for SRB but it indicates that anaerobic bacteria are present in the sample being tested. Note that these types of events often also occur in the DN-BART if both are set up for the WS. Anaerobic bacteria are recognized by the dead organics (DO) reaction in the HAB-BART.

9.34 SLYM-BART REACTION PATTERNS

Slime-forming bacteria are composed of those bacteria able to generate excessive amounts of slime during growth. This slime coats all of the cells and it can also permeate into the water making it viscous with biocolloids. For the user of the water, the water may appear cloudy and to some even “slippy.” If the water becomes infested with these bacteria then slime coatings tend to appear on walls, in pipes, and particularly on drain openings. In general while there is a loss in the clarity of the water, there is commonly neither major color changes nor are there strong odors or tastes. All of these problems can however develop once the growth matures and even thread-like slimes may be seen growing in the water.

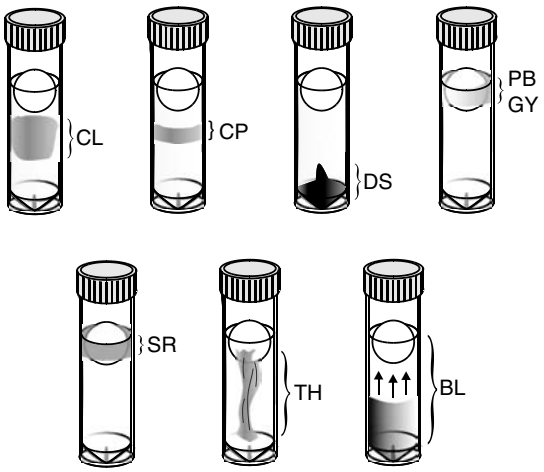

FIGURE 9.34 Standard reaction pattern locations for the SLYM-BART tester.

To detect the slime-forming bacteria, SLYM-BART testers employ a rich culture medium that causes very rapid growth. Using this medium the SLYM-BART tester can exhibit seven different reactions (Figure 9.34) with the most dominant first reaction being CL (cloudy growth). This normally first begin at the redox front roughly half way down the tester and may sometimes resemble lateral cloudy plates (CP) floating in the culturing medium for 1–4 h before dispersing into a clouded reaction. Dense slimes (DS) can form in the basal third of the tester and commonly these follow the CL reaction when they occur. These dense slimes do have an integrity that can be observed by titling the tester by no more than 20° and noticing that the gelled mass moves with the tilting of the tester and not with the WL. It is fairly common for a slime ring (SR) reaction to form around the BART ball and this again is a secondary reaction. Usually the SR initial glistens (reflective) and has a white to light beige color. This color can intensify overtime and occasionally will turn violet. On some occasions, the bacterial growth will take on a thread-like quality (TH) interconnecting the BART ball to the basal cone with one to four slime corridors. These TH growths may last for hours to several days. If there is a bacterial community incorporating pseudomonad and enteric bacteria then a terminal reaction can occur where the culturing medium takes on a black color (BL, blackened liquid). Occasionally pseudomonad bacteria will cause a pale blue (PB) or green yellow (GY) reaction mainly around the ball.

9.35 HAB-BART REACTION PATTERNS

Of all the bacterial groups, the heterotrophic bacteria are the masters of degrading specific organics [such as petroleum hydrocarbons, natural gases, solvents, and organic leachates from landfill sites]. This method focuses on the ability of the heterotrophic bacteria to shift the HAB-BART tester from an OX to a RD condition.

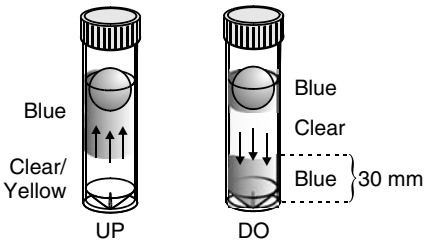

FIGURE 9.35 Standard reaction pattern locations for the HAB-BART tester.

When this happens then the methylene blue changes color from blue to clear. Aerobic (oxygen loving) bacteria will cause this to happen first in the base of the tester while anaerobic (oxygen hating) bacteria will tend to cause reduction higher up in the tester and then move the zone down the length of the tester. This appears odd for the anaerobic bacteria to begin activities under the BART ball rather than in the base. It appears that the anaerobes actually block the diffusion of oxygen down the tester by form a biocolloidal barrier just below the ball.

Therefore, there are two reaction patterns commonly seen in the HAB-BART tester. Initially once the methylene blue has been mixed following the standard protocol then the tester will have an even blue color that may show some discoloration in the basal cone as the selective medium pellet dissolves and diffuses. This may give a yellow or yellowish green color within the cone. In Figure 9.35 the left-hand diagram shows the UP (reduction of the blue to clear moves upwards from the basal cone) reaction which is typical of bacterial communities that are predominantly functioning aerobically. To the right is the DO (reduction of the blue to clear moves downwards from just below the BART ball and upwards around the ball) reaction which indicates that the dominant bacterial communities are anaerobes. A DO reaction tends to produce clear mobile clouds in the blue solution just below the ball but indications of a positive occurs when the reduction zone descends in an even lateral manner to 30 mm above the base of the tester. Where there is intense aerobic (UP) reactions, the slime twisters may be seen in the culturing medium above the reduction front. These slime twisters appear to have the ability to move oxygen from the headspace around the ball and down to the reduction front in an active rotational manner.

9.36 DN-BART REACTION PATTERNS

Nitrates are a serious concern in drinking water because of the risks to the very young and the very old when consuming such waters. Nitrates are primarily produced under OX conditions by the nitrifying bacteria converting ammonium to nitrates. Ammonium products are generated through the bacteriological anaerobic decomposition of proteins in organic material. When this ammonia moves into an aerobic then nitrates are produced by bacterial action. When these nitrates now enter an anaerobic or reductive environment again then denitrifying bacteria can now

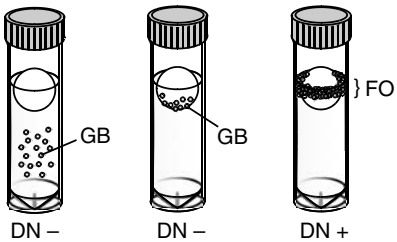

FIGURE 9.36 Standard reaction pattern locations for the DN-BART tester.

breakdown the nitrate primarily to nitrite and then on down to dinitrogen gas. These denitrifiers are major players in the movement of nitrogen gas back into the atmosphere!

Figure 9.36 illustrates that the DN-BART has a single reaction of significance which is illustrated above as a FO ring of nitrogen gas bubbles (GB) around the BART ball. This reaction can only be considered positive when there is a ring of GBs forming at least three quarters of a FO ring around the BART ball. Gas bubbles attached to the underside of the ball or bubbles on the wall of the tester should be discounted since these do not necessarily indicate denitrification is happening. Denitrifiers have the potential to remediate nitrate challenges in water but only when the water again becomes RD or anaerobic and there is a significant level of organics in the water. Under OX conditions, the denitrifying bacteria are not very efficient at reducing nitrates and so conditions can exist where nitrate concentrations are significant along with denitrifiers but the nitrates are not degraded.

9.37 N-BART REACTION PATTERNS

This BART tester is a little different to the standard BART testers that have to be observed periodically for a recognized reaction or activity. This test is incubated for a standard period of 5 days after which it is tested for the presence of nitrites. The culture medium contains only ammonium-forms of nitrogen and no oxidized forms such as nitrites or nitrates. The incubation period is long enough to allow active nitrifying bacteria in the sample time to oxidize at least some of the ammoniacal compounds to nitrite and then nitrate. In natural samples of water, there is a possibility that the denitrifying bacteria might also be present. In this case then the nitrate will be quickly reduced to nitrite again. This test relies on the detection of nitrite and not nitrate because it has been found that nitrate presence is too transitory to allow use as the indicator chemical for the activity of nitrifying bacteria. Nitrite on the other hand appears close during nitrification and again as the denitrifiers become active.

Figure 9.37 shows that there are three reaction patterns that are shown in the diagram above. These reactions are generated during the 5-days incubation at room temperature and determined by the level of nitrite recorded. It is important to follow the standard procedures associated with replacing the BART IC with a reaction cap.

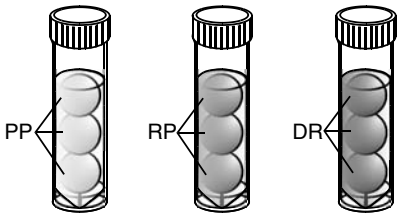

FIGURE 9.37 Standard reaction pattern locations for the N-BART tester.

The reactions are: PP, partially pink which means that only half the three BART balls used in this test are pink, while the remainder of the ball surfaces remains white and the culturing medium remains colorless; higher levels of nitrite generated by the nitrifiers cause a red-pink (RP) reduction in which the BART balls are now coated red and the culturing medium is now a pink color; if very high levels of nitrites causes a DR, dark red reaction to be observed both on the BART balls and in the culturing medium. Note that this test cannot be applied to WSs having >3 ppm of nitrites in the water since this could generate false positives.

9.38 ALGE-BART REACTION PATTERNS

Figure 9.38 shows the six common reactions that can occur in the ALGE-BART tester and these are displayed above in three rows of two reactions. This test differs from the other testers in that incubation involves laying the tester at a 5° angle from the horizontal and applying illumination in the form of natural or artificial light in a continuous manner. This tester uses to woven fabrics placed on the walls of the tester to allow micro-algae to attach to, and grow, commonly on the white textile as colored patches of growth. Commonly, these growths are some shade of green

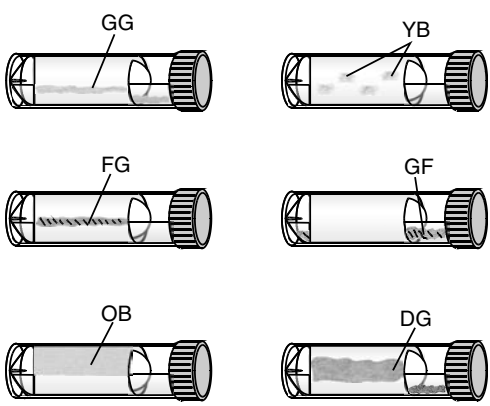

FIGURE 9.38 Standard reaction pattern locations for the ALGE-BART tester.

ranging from lime-green through to grass green, dark green, blue green and even brown, yellow, red, or purple.

Figure 9.38 includes six of the major reactions that can be observed. Each reaction has distinct characteristics that are summarized below:

GG shows a growth that is grass green (GG) in color and lies at or just below the water line. This line has a smooth edge—*Chlamydomonas*; yellow beige colors are generated as small but distinctive patches of the textile—*Scenedesmus*.

Fuzzy green (FG) growth that forms as patches on the textile generally below the water line—*Chlorophyceae*.

Floating green growth (GF) is normally seen in the medium or on the floor of the tester—*Chlorella*.

Some algae generate orange and beige (OB) patches of growth usually observed at or above the water line in the textile. Occasionally red are also observed—Diatoms and Desmids.

These algae generate a dark green or blue green growth that darkened commonly to black. These growths occur at the water line and are thicker in the textile—*Cynaobacteriaceae* or blue-green algae.

Algae occur in wells and groundwater under two possible conditions commonly. First if the well is directly exposed to the light (e.g., not capped) and the water column is high enough to be directly exposed to the light. Second would be particularly in fractured bedrock that has direct connection to surface waters where these algae are growing. Porous media will tend to filter out the algae and at least partially prevent their entry into the water well. It should be recognized that if the groundwater in the well has significant nitrogen and phosphorus components then this would increase the likelihood of growth under either of the above conditions.

9.39 APB-BART REACTION PATTERNS

Microbiologically influenced corrosion has traditionally been connected to the activities of SRB, but today there is a growing body of concern that many anaerobic bacteria generate organic acids that lead to corrosion. While these acids are not as aggressive as the inorganic acids, such as hydrochloric, they do sufficiently lower the pH to affect the structural integrity of the metal alloys. Of these alloys, it is possibly the aluminum-based metals that are the most vulnerable to pitting and perforation by these acid-producing bacteria (APB). Today the management of corrosion now often includes both the SRB and the APB tests to minimize the risk. One additional concern that can be linked to APB is the corrosion of concretes that tend to become more vulnerable under the more acidic conditions created by the APB.

In the APB-BART test, the critical reaction occurs from 2 to 10 mm above the baseline where the basal cone fuses into the side alls of the tester (see Figure 9.39).

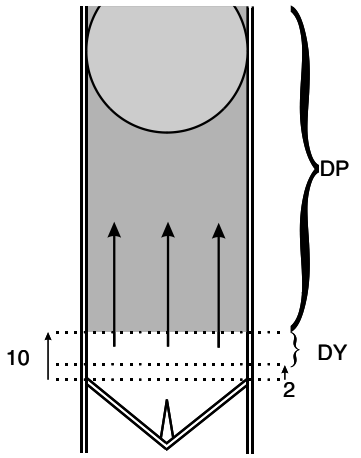

FIGURE 9.39 Standard reaction pattern locations for the APB-BART tester.

Here, the position of this zone is shown with changes in color occurring from a relatively clear purple to a dirty yellow (DY) as the test goes positive for the presence of active APB.

This APB-BART test begins with the WS forming a clear purple to dark purple (DP) with an intensity that is affected by the chemicals present in the sample. Initially when the test is started, the culturing fluids will appear to vary through shades of blue to purple. A positive detection of activity is declared when a DY color can be observed in the zones from 2 to 10 mm above the start of the wall of the tester. This color will gradually move to a yellow in the basal cone and to lighter shades of brown to orange in the culturing medium. It may take several days for the whole of the culturing medium to turn to a DY and so the time lapse for declaration of a positive detection should be based when this narrow 8 mm zone close to the basal cone has shifted to a DY. The rate at which the DY extends upwards into the body of the culturing fluids is not relevant to the time lapse.

9.40 RELATIONSHIP OF TIME LAPSE TO POPULATION

Almost all of the BART testers (except for the N-BART) do use the activity levels for the selected bacteria in the sample as the prime factor to determine the population size. The premise here is that if the selected bacteria are not active, or not present, then they are not significant components in the predicted active cells (p.a.c.) population. To convert the time lapse (as a measure of the activity of the targeted microbes) to the population a standard unit is used. This is the p.a.c./mL. This differs from the conventional colony forming units per mL (cfu/mL) in that the latter methods are a direct count of the colonies that have formed under the conditions stipulated. This count is restricted to only those cells (or clumps of cells) that have been able to grow on the surface of the agar and generate a visible (countable)

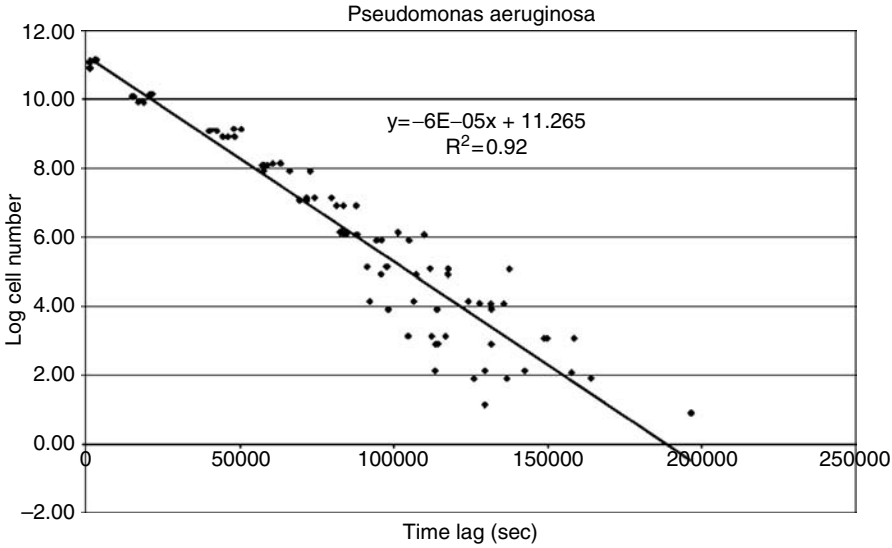

FIGURE 9.40 Graphical generic link between time lapse referred to as time lag in the figure (*x*-axis) and log of the population size (*y*-axis).

colony. This is a serious restriction since many bacteria are not able to grow on such agar surfaces and therefore are eliminated from such counts. For the BART tester generating a time lapse, there are a rich variety of environments within which the bacteria can grow and register an activity or reaction. This method is therefore more comprehensive in its form and captured a wider spectrum of bacteria that are then enumerated as p.a.c./mL using a conversion equation that is used in QuickPop software version 3.

Figure 9.40 shows a theoretical graph illustrating the inverse relationship in a generic manner between the time lapse and the population of bacteria that are active within the conditions established in the BART tester. For this example, the time lag lies on the *x*-axis while the *y*-axis gives the population size in p.a.c./mL. In the BART tester, the prime mandate relates to the fact that it will be only the active cells that adapt to, and become active, in the culturing medium that will cause the reactions to be generated after a period of time (time lag) that would be inversely linked to the active population size.

9.41 ORP RELATIONSHIP TO IRB-BART REACTIONS

Oxidation–reduction potentials vary in the BART tester from commonly oxidative around the BART ball to very reductive in the bottom of the tester where the selective chemicals are diffusing upwards into the sample. Many of the IRB-BART reactions have been found to link to the probable ORP value of the original site

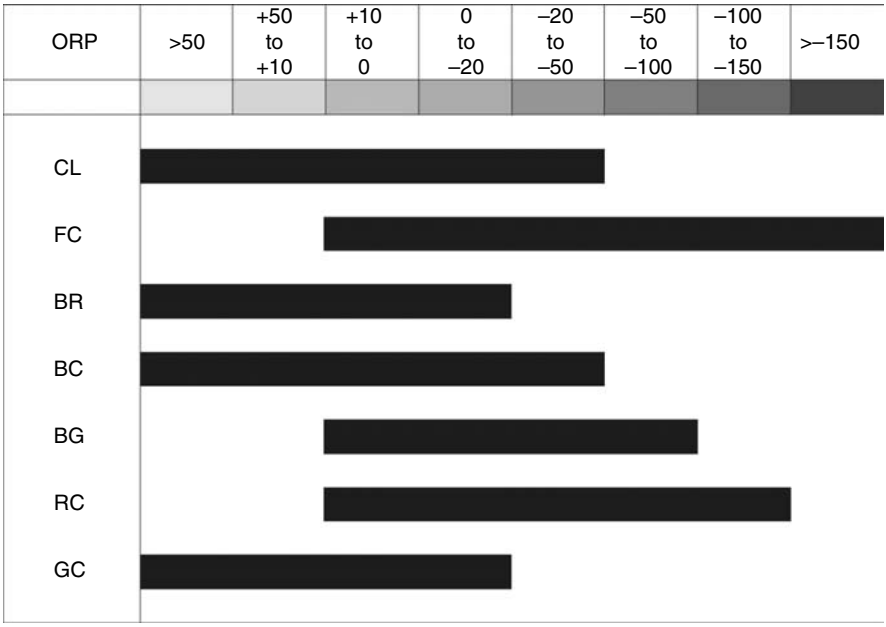

FIGURE 9.41 Relationship of IRB-BART reactions to the oxidation–reduction potential value.

where the IRB were active before the sampling was taken. For example, the generation of FO particularly as the first recognized reaction that then generates the time lapse would mean that the IRB were active in a RD environment. Brown ring reactions tend, on the other hand, to occur where conditions are OX and ferric-iron is being accumulated within the SR.

Figure 9.41 uses a series of lateral bar histograms to show the OX and/or RD conditions (expressed as ORP) under which these reactions are most likely to occur in a given sample. These histograms therefore show the relationship between each of the IRB-BART reaction types (along the *y*-axis) and the ORP as the *x*-axis. Each reaction type is shown laterally with range over which activity can occur given as a single black box. Extending over the range where this reaction type can occur. A summary of the reactions is listed below:

CL is a clouded reaction which generally occurs throughout the tester and is most easily observed by holding the tester up to a bright light and the sample solution would be seen to be cloudy.

FO is a reaction that occurs when bubbles specifically collect around the BART ball. Bubbles on the wall of the vial or under the ball should be ignored for this reaction.

BR is formed at the sample surface and around the BART ball. Commonly slime forms before the ring goes brown but on some occasions the sample solution will go orange to red in color before a brown ring forms.

BC occurs when the whole of the culturing sample in the tester becomes brown in color. It usually follows the CL reaction under more OX conditions.

BG occurs in the base of the IRB-BART tester as a brown gelled formation often with the solution above becoming clear and without color.

RC that occurs throughout the sample solution and commonly may become a little turbid. More associated with RD conditions and the presence of significant numbers of enteric and pseudomonad bacteria.

GC is a green-clouding of the sample solution that gradually darkens and may get more clouded. This indicates more OX conditions and a dominance of pseudomonad bacteria.

9.42 ORP RELATIONSHIP TO SRB-BART REACTIONS

Oxidation–reduction potentials vary in the BART tester from commonly OX around the BART ball to very RD in the bottom of the tester where the selective chemicals are diffusing upwards into the sample (Figure 9.42). There are only two reactions of significance in the SRB-BART have been found to link to the probable ORP value of the original site where the SRB were active before the sampling was taken. Sulfate-reducing bacteria are major bacteria associated with corrosion and plugging problems in water wells as well as a principal factor in the declines in water quality.

These reactions are summarized as being associated with very RD as the black base (BB) reaction, while the SRB can be involved with aerobic bacteria in a black reaction that occurs primarily around the submerged parts of the BART ball. This reaction is a black top (BT) reaction that occurs primarily around the ball at the top of the BART tester.

In cases where the SRB-BART is only observed on a daily basis, it can be that both reactions would have occurred during the 24-h period between observations.

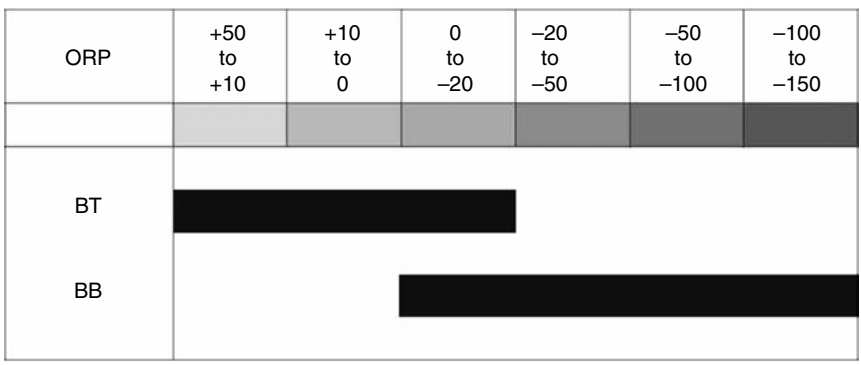

FIGURE 9.42 Relationship of SRB-BART reactions to the original sample oxidation–reduction potential values.

This is called a black all (BA) reaction but is defaulted to a BB reaction since commonly the BB reaction precedes the BT reaction.

Treatments of biofouling water wells whether for regeneration or preventative maintenance (PM) can use type of SRB-BART reaction on the samples to define some aspects of the treatment. If the reaction is a BT then it means that the SRB are more vulnerable to treatments since they are cohabiting with aerobic bacteria. This means that the treatment needs to include processes that would strip off the biomass (e.g., biostatic detergent). Additionally, adjustments to the pH (commonly going acid) will destroy the biomass and application of heat would raise the temperature to above the "comfortable" range for the bacterial biomass.

9.43 EFFECTS OF SAMPLING DURING PUMPING ON BIOMASS CHARACTERISTICS

When extraction water wells are turned on and go into a pumping cycle then groundwater moves towards the pump in a sequence that reflects the manner in which water is moving towards the well, into the pump. Figure 9.43 shows a vertical section through a well indicating the main passage routes (conduits, as arrows) though which groundwater moves into the pumping well (PW) towards the pump. Dotted line shows the redox front and shaded region shows where the bulk of the biomass is positioned relative to the well. Vertical arrow (W) shows pumped water from well. Samples taken from the water as it is being pumped will therefore tend to reflect the characteristics at the site from which the water first began moving towards the well. During the processes of moving towards the active well then there would be

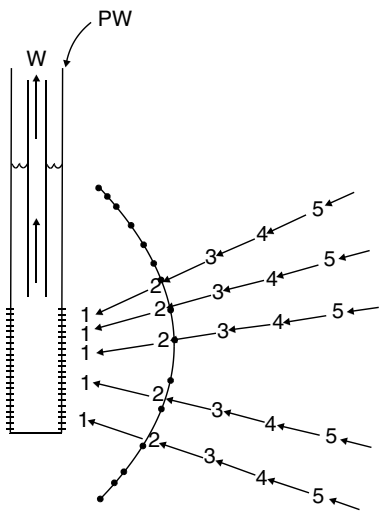

FIGURE 9.43 Sequence diagram indicating changes in sample location origin during a continuously active pumping of a water well.

some intermixing between the various sources of water now moving in a sequential manner towards the pump and discharge from the well. Sampling at a discharge point close to the well head should therefore, when treated using the BART testers, then reflect the original sites from which the water waster sample was first moved towards the well.

Figure 9.43 illustrates this where numbers 1–5 represent a sampling sequence from sites around the well (W). Groundwater is being pumped (vertical arrow) in sequence from the producing well (PW). The order in which the samples are coming from the well is shown in a numerical order from 1 to 5 where 1 would be the first sample extracting water close to the well, while 5 would be the furthest point from which groundwater was taken. The numbers therefore indicate the approximate position for each of the five samples taken. These samples may reflect the position of different bacterial communities around the well. For example, sample location 1 was the first water pumped and dominated by IRB. Sample location 2 in this case is a transitional sample dominated by both IRB and HAB. Location 3 sample was extracted from the major active biomass at the redox front and was found to be dominated by HAB and SLYM. Farther out at location 4 which was on the RD side of the redox front; it was dominated with SLYM and SRB. In the final sample taken from location 5 which was on the RD side of the redox front; this sample was found to include SRB and various anaerobic bacteria including DN and HAB. Here the HAB now gave a DO reaction while previous reactions were all UP.

9.44 LOCATION OF BIOMASS SHIFTING DURING PLUGGING OF WELL

During the life span of a water well that is being impacted by biofouling then the biomass that is principally causing the problems will be moving primarily in response to the location of the redox front. The focus of the biomass moves as a reflection of the groundwater flow, the level of nutrients moving in with groundwater, and the location of the redox front.

Figure 9.44 incorporates four vertical sections of producing water wells (PWW) as they age causing biomass to grow and also possibly move. This growth will change the movement of groundwater towards to wall and, as the biomass begins to plug the porous or fractured conduits inside the well, begins to plug (lose Q/s). This figure therefore shows the sequence of biofouling caused by the biomass growing around the well. The figure shows a newly installed and developed well (1). At this stage the well is still at 100% of the postdevelopment Q/s. In the next stage (2), the well is now starting to foul and lose its original Q/s. Stage (3) now shows the well that has now lost 50% of its specific capacity, Q/s. At this stage, there is now a degraded water quality primarily caused by sloughing from the biomass which includes bioaccumulated metals (such as ferric-iron). Once the well now becomes virtually plugged (4), there is a $>90\%$ loss in Q/s. During this plugging and loss in the Q/s, the center of activity for the biomass moves depending upon the various factors such as plugging locations and the movement of the redox fronts.

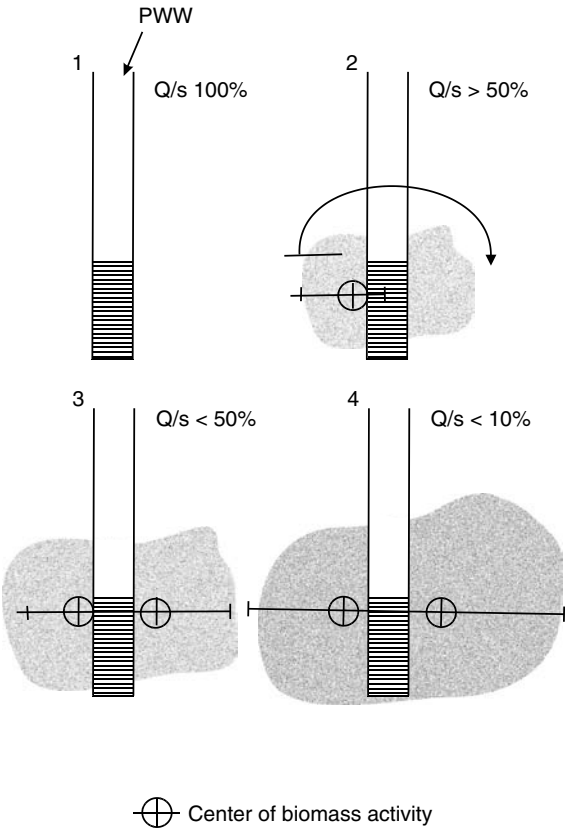

FIGURE 9.44 Illustration of the biomass center of activity's evolution during plugging water wells.

This movement in the center of activity for the biomass is shown in the wells (vertical section) before (1) and during the process of biofouling. At each stage the focus of activity by a circle is divided into quadrants from which extends a lateral line showing the extent of the sphere of influence exerted by the biomass.

9.45 PRIMARY EFFECT OF WATER WELL TREATMENTS

This illustration of an extraction WW that has become heavily plugged with biomass. Figure 9.45 illustrates treatments of the well by showing four vertical sections of the plugging water well as a result of a treatment involving sequences including shock (SK) and subsequent disruption (DR) and dispersion (DS) of the impacted biomass within the well. This figure shows four stages in the successful rehabilitation of the well with the effective removal of the plugging biomass and the return of the well closer to its original Q/s.

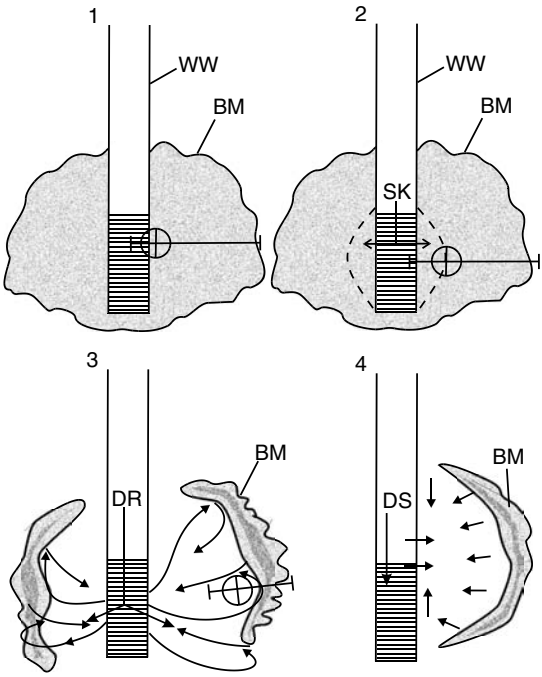

FIGURE 9.45 Impact of successful regeneration of plugged water wells on the location of the surviving biomass.

In the figure, the first WW diagram 1 shows the biomass (BM) clustered tightly around the slotted region of the well. Here the center of activity (circle with quadrants) remains fairly close to the well. The first treatment is applied as a SK to the biomass shown in diagram 2. This disrupts the biomass causing the center of activity to move further away from the borehole. Further treatments shown in diagram 3 involving DR and dispersion (DS) applied through the borehole are shown as arrows. These additional treatments now cause much of the biomass to collapse and the center of activity now moves further away from the well. Once the treatment has been completed then there is, the treatment has been successful, a considerable area of cleaned surfaces closer to the well. The surviving biomass (and there will always be one some distance from the well) will attach and grow on these pristine surfaces cleaned by the treatment (diagram 4). While the reinfestation of the well is occurring inside the treated zone, there are a number of events that should be considered. First this surviving biomass as it moves in and attaches to the freshly cleaned surfaces will also impart high populations of bacteria into the water. If sampling is done immediately after even a very successful treatment then it can be expected that bacteriological count performed immediately after treatment could be very high. Second it takes a number of weeks for these infesting bacteria to attach and stabilize on the surfaces around the well and posttreatment sampling should wait ideally until 8 weeks after the treatment. Generally stabilization occurs in the first 2

weeks and useful testing can be conducted after as short a time as 4 weeks if there is urgency on the client's part for posttreatment evaluation of the treatment. Third even if the treatment has been totally successful the conditions exist for reinfestation of that well and PM.

9.46 MECHANISMS FOR BACTERIOLOGICALLY INFLUENCED INFESTATION OF WATER WELLS

Figure 9.46 illustrates the manner in which successfully regeneration of water wells do not become compromised by infestations from the surviving microbial biomass from the fringes of the effective treatment zone. There are four stages (1, 2, 3, and 4) showing the chronology of events from prior to treatment through to when the well enters a stable state after reinfestation. Each diagram is shown as a vertical section down the well (W) with the current position of RF (as a dotted line) and biomass (B, shaded indicating density of growth). Diagram 1 shows the destruction of the biomass closer to the well but with dead organisms (DO) scattered throughout that impacted zone. Diagram 2 now shows the initial infestation and colonization (C) of the cleaned surfaces within the treatment zone. Here there is now a large expansion in biomass some of which sloughs (S) off easily into the flowing groundwater

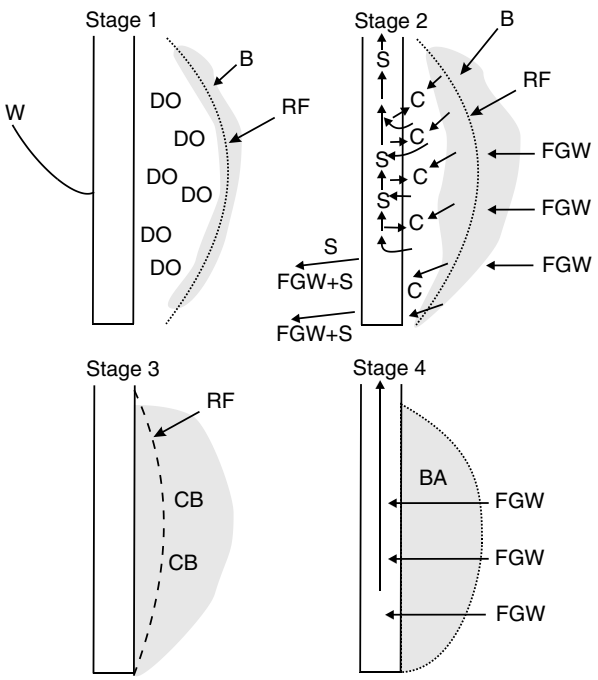

FIGURE 9.46 Chronological sequence of growth that is likely to occur in a biomass that has survived rehabilitation.

(FGW). Diagram 3 involves an attachment (A) and compression of the biomass so that the voids within the impacted porous media now become open and Q/s returns to a stable and acceptable level. In the last diagram 4, the biomass (BA) has now attached tightly to surfaces with very little sloughing (sheering) of cells in the FGW. The well is now producing closer to its original Q/s and the colonising bacteria (CB) in the biomass are preoccupied with growth and garnering nutrients from the FGW but do not have the biomass volume to significantly affect the well.

9.47 ZONES OF INTERROGATION PROJECTION (ZIP I)

Biomass is not only clustered in the borehole but also occurs around the slots or perforations, in the immediate fractures and porous media just outside the well and further out where it merges with the indigenous communities occupying the groundwater in the aquifer. Essentially all of this growth can be viewed to be as a series of communities forming effectively cylinders of slime in, and around the well. Groundwater permeating through this biomass may end up entering the well as produced water from the well that has passed through these natural filters. If water wells are to be regenerated or serviced to maintain production then the location, and form, of these concentric slime cylinders forming the biomass. Zones of interrogation (ZIP) have been used as a geological term that can be applied to define the composition of the biomass as it is affected by the "cylinders of slime." Figure 9.47 through Figure 9.49 are examples of different forms of ZIP diagrams

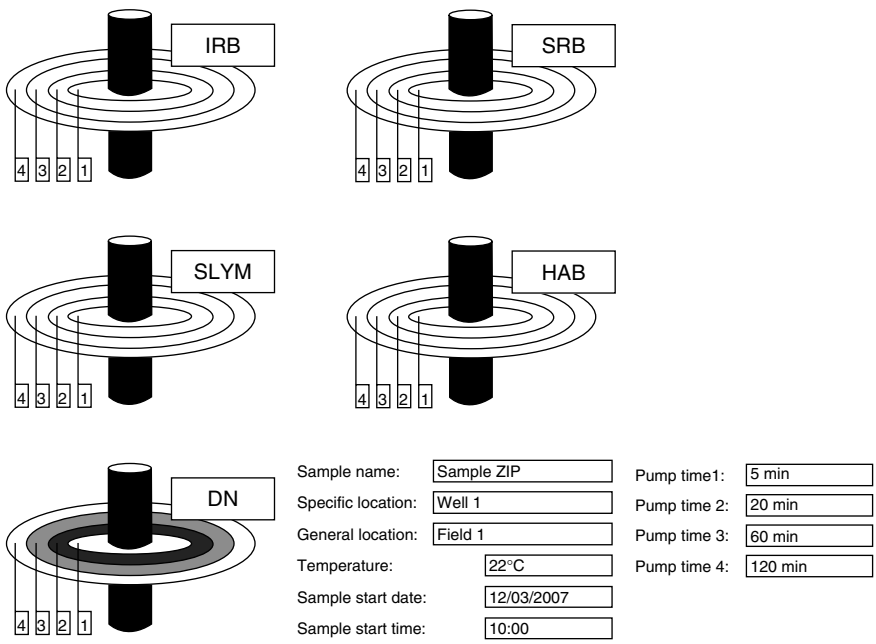

FIGURE 9.47 Typical zones of interrogation projection for freshly developed water wells that are not significantly biofouled.

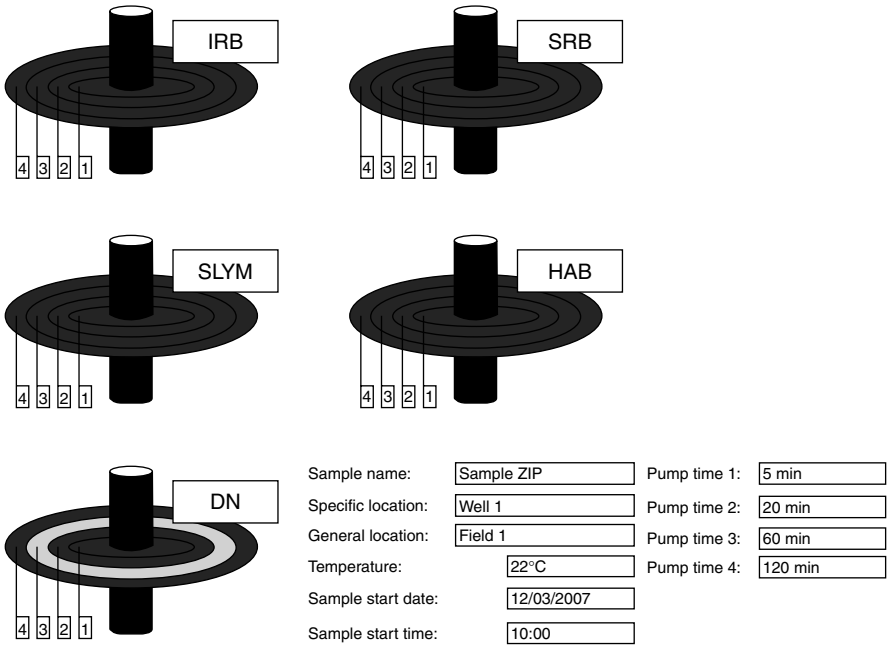

FIGURE 9.48 Typical zones of interrogation projection for a badly biofouled water well.

that can be generated for water wells. These ZIPs may now be used to determine the nature of the biofouling biomass around the well. These have been generated using a sequence of pumped samples over a specified period of time (e.g., 3 h). During that pumping, samples at timed sequences are taken for BART testing that then can be used to interpret the positions of the various bacteria around the well.

In this ZIP the water well borehole is represented by four concentric rings representing the four pump test samples tested with the location being shown with the closest ring being the first pumped sample and the outermost ring the fourth tested pump sample. Five bacterial groups were tested and their activity is given in shaded rings. For the bacterial groups (descending order) examined as IRB-, SRB-, HAB-, SLYM-, and DN only DN was detected in the second and third pumped sample. No bacteria were detected using the BART testers for IRB-, SRB-, HAB-, SLYM-bacteria. This well was not significantly biofouled by bacteria likely to cause plugging or corrosion. Zones of interrogation programs form a part of the BART-SOFT interpretation and archiving package that can be downloaded from the web site www.dbi.ca.

9.48 ZONES OF INTERROGATION PROJECTION (ZIP II)

When water wells become badly biofouled with plugging and the Q/s had lost 80% with the water quality degenerated to unacceptable levels, then the ZIP from such a well would indicate high levels of bacterial activity. In this example, there were four

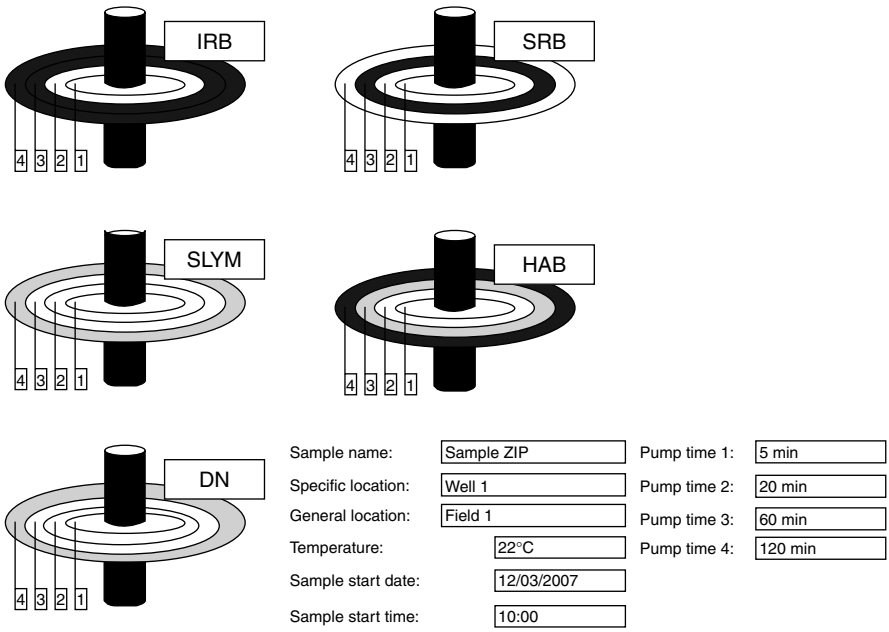

FIGURE 9.49 Typical zones of interrogation projection for a biofouled water well that has been successfully regenerated at least 40% of the Q/s towards postdevelopment values.

pumped test samples tested and the water was found to contain very active communities of IRB- (plugging), SRB- (corrosion), HAB- (turbid waters), SLYM- (slime type of plugging) while DN were located in two zones around the well. The more intense the shading then the greater is the level of bacterial activity. Here, the well was now becoming terminally infested with all of these bacterial communities as at sites except the denitrifying bacteria which were located as being very active in two of the zones but less active in the third in the middle zone.

9.49 ZONES OF INTERROGATION PROJECTION (ZIP III)

When a well has been successfully regenerated (Q/s returning by at least 40% closer to the original postdevelopment 100%), one of the common effects after treatment and when stability has returned to the biomass, there is less bacterial activity in the earlier pumped samples tested using the BART-ZIP system.

In this illustration of a ZIP, it can be seen that all five bacteria groups have been suppressed around the well (particularly pumped samples 1 and 2 that were the first ones taken for the ZIP testing), but bacteria are still active in the later four of the six pumped samples that were tested. This would indicate that while the treatment applied downhole did have an impact close to the well but there remains an active bacterial population further away around the borehole. There is with this example

a high probability of infestations and biofouling again occurring unless kept in check by regular effective PM servicing of the impacted well.

9.50 RELATIONSHIP BETWEEN BACTERIAL ACTIVITY AND SPECIFIC CAPACITY IN BIOFOULING WATER WELLS

Bacterial activities around water wells can form a reflection or even a prediction of the types of biofouling that are occurring in those wells. Figure 9.50 shows four graphs that illustrate different ways in which these bacterial activities can impact on the Q/s (specific capacity). Each of these four graphs have an *x*-axis which shows four time intervals during the history of the well. For the *y*-axis, this is a generic and may reflect relative populations (higher populations being further up the axis) and also the Q/s in which the upper point of the axis would be 100% of the original specific capacity and the base would be a zero value. Each graph operates under different set of conditions.

The first graph (upper left, 1) illustrates an ideal condition when the water well is not being impacted by any significant bacteria activity (SRB, dashed line and IRB, dotted line) and the Q/s (continuous line) remains unaffected over 40 years (*x*-axis). In the graph set upper right (2) there is a rapid decline in Q/s primarily resulting from primary IRB and secondary SRB activities that occurred as a series of pulses over 4 year (*x*-axis) causing the well to fail. The graph presented lower left (3) now shows conditions where corrosion was severe over a 4-year time frame (*x*-axis) with failure occurring when the pumping equipment failed. In this example the SRB now dominated the bacterial activity primarily with BT reactions indicating that the SRB were active in associations with (primarily) aerobic heterotrophs and slime formers. Note that the SRB are designated as BT or BB reactions. This will suffered from

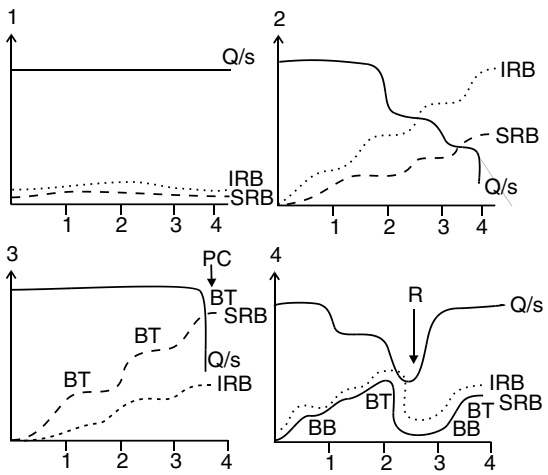

FIGURE 9.50 Graphical presentation of the shifts in specific capacity (Q/s) and microbiological activity (time lapse) in biofouling water wells.

a sudden and dramatic loss in production due to corrosion induced failure of the pumps. Lower right is the final graph (4) in which there is a series of pulses of IRB and SRB activity functioning in harmony leading to triggered stepwise failure of the Q/s. In year 3 the well was subjected to a successful regeneration treatment (RT; R, vertical arrow) which led to a suppression of the bacterial activities and a recovery in the Q/s.

9.51 MOVEMENT OF THE REDOX FRONT AROUND A WELL SUFFERING MICROBIOLOGICALLY INFLUENCED PLUGGING

When water wells begin production they will automatically exert an influence on the local environment around the borehole. These effects are a complex set of interactions that involve the draw down of air (oxygen) inside and possibly outside the casing, increasing levels of turbulence in the groundwater as it approaches the well, and the effects of electrical charges emanating from the motors. All of these factors will influence the locations of any microorganisms that might be active in, or around, the well. These factors also affect the location of the redox front as the well ages which would then in turn affect the precise location of the biomass active at those mobile redox fronts. A very "mobile" redox front is likely to have a "smearing" effect on the location of the biomass activity associated with that front. This would mean that the biomass would take longer to cause plugging and severe problems for the water wells performance (good news) and that, when these plugging impacts occur, they will be more difficult to treat because of the extended locations involved in the treatment (bad news).

Figure 9.51 illustrates the vertical section of producing water well (PWW) in which the position of the redox front is shown (dashed lines) in relation to the

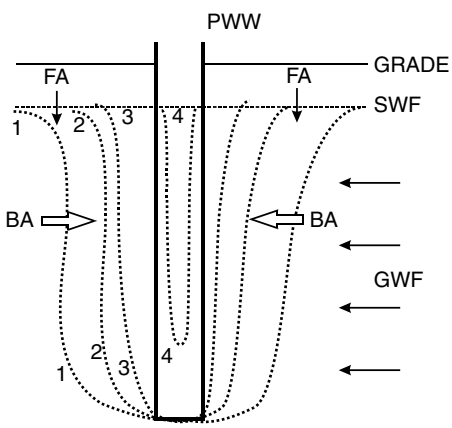

FIGURE 9.51 Diagrammatic presentation of the movement of the redox front and associated biomass in biofouling wells.

well itself. Groundwater flow is shown as arrows and static water front (SWF) is presented as a continuous line. This illustration shows where, overtime, the redox front moves to from start when the well was freshly completed but not in production (1). When the well first comes into production (2), there would be a low level of bacterial activity and the redox front may be positioned from the PWW to a greater distance in the downstream direction. Bacterial activity (BA) now causes the redox front to shrink (3) while the fungal (mold) activity (FA) at the SWL will begin to block the downward movement of oxygen into the formation. Once the well is heavily biofouled (4) then the redox front would be close to the well and respiration activities would be more dependent upon alternate electron acceptors, such as nitrate, sulfate, and ferrous iron.

9.52 TREATMENT IMPACTS ON BIOFOULING WATER WELL

Like all other "living" things, water wells have the equivalent of a heart beat as the signal of life. Unlike all living things that pump blood and hence have a heart beat, water wells are complexes of living systems through which water is being forcibly passed by some construct such as pumping, gravity feed or the generation of hydrostatic pressure. Here unlike the heart beat that is a positive indication of biological health; water wells generally signal the health of the biomass as being a potential sickness to the well. This is because the living growing biomass now impacts upon the movement of groundwater towards, or away from, the well. Symptoms of these impacts are a falling specific capacity (Q/s), increases in the draw down, degenerating water quality, and increasing evidence of biological activity.

Figure 9.52 is a graph where the *x*-axis is time (years) and the *y*-axis is the Q/s given as a percentage of the original specific capacity (right-side axis, line) and microbial populations (left-side, dotted line as log p.a.c./mL). The first event (1) is an increase in the bacteriological activity followed by a stepwise fall in the specific capacity (Q/s). This decline may be corrected by regeneration (R) which would be a treatment to recover the Q/s and suppress the bacterial activities. The well returns

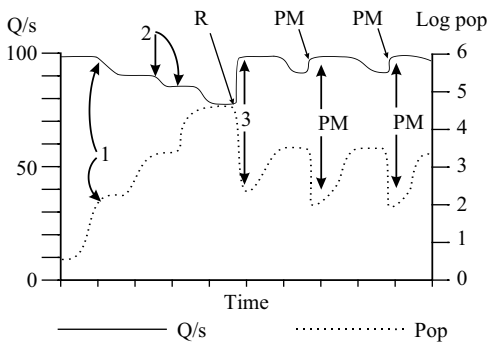

FIGURE 9.52 Sequence diagram for the sustainable management of biofouled water wells through sequential regeneration and preventative maintenance.

to its original capacity (3) and the bacterial activity is suppressed. However, PM servicing of the well is needed to keep the biofouling in check and maintain the Q/s. When properly applied then the PM of the well following regeneration allowed the well to become sustainable.

9.53 VIDEO-CAMERA LOGGING OF BIOFOULED WATER WELLS

One inspection tool that is very important in water well monitoring is the video camera with lights and lenses that can point down or to the side to inspect the well casing, welds, slots, and perforations. Already adopted as an inspection tool for determining mechanical and structural failures, the camera also has an important role to play in examining the well for evidence of significant biofouling. As the camera moves down the well then there are a number of clues that biofouling is occurring. In one word, this can be summarized as "growth" occurring on the casing walls, in the water, the slots and in the fractures if these are visible. There is also one word "caution" since it is important to recognize that the camera can only see that is visible to the lens. What is not visible to the lens is all of the biomass growing away from view outside the well in the porous media and fractures. Caution should be followed in that a clean well by VC logging and inspection does not necessarily mean that the well free from biofouling challenges. Only that the well is free of any obvious symptoms!

Figure 9.53 is a composite illustration of the principle forms of biofouling that can be observed through VC logging of a vertical extraction well. These forms are

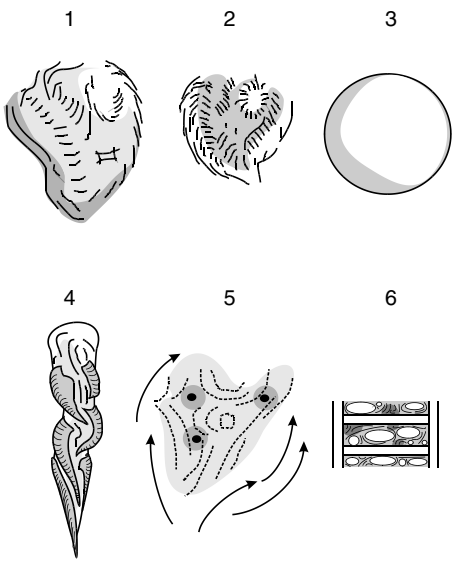

FIGURE 9.53 Schematic of the key elements in downhole video-camera logging.

described as follows: (1) slime-like growth commonly glistening; (2) encrustation-type of growth which are commonly matted and complex in surface structures and often brittle (breaking off easily when touched); (3) nodular has a dome-like structure that is uniform and hard or tuberculated growths are raised from the surface and show a ridge at the outer extensions and usually not reflective; (4) rusticle-like encrustations that appear to hang down inside the well but are attached for much of the length to the well surfaces; (5) suspended colloids that appear ahead of the camera to be almost "glued" in their position (due to the BCs within the well water); and (6) covert growths inside and beyond the slots or perforation. These are difficult to observe but these can cause blockage of the voids with biomass and deposited materials (white crystalline material is most likely to be carbonates or possibly sulfates). Because these wells are commonly on the oxidative side of the redox front, there may be very significant accumulations of ferric rich (orange-red-brown) materials. If the well is more reductive then black or grey may dominate the colors due to the accumulation of iron sulfides and carbonates.

9.54 METHANE (NATURAL GAS) ERUPTIONS IN WATER WELLS

There are many stories of owners of water wells getting gas coming out with the product water. Sometimes this gas production occurs with such regularity that the well is capped and used as a gas well as well as a water well! Commonly, it might be believed that the water well has intercepts a vein of NG but this may not be the case. On the far side of the cylinders of slime biomass around water wells it is not uncommon to find methane-producing bacteria active in the very reductive outer regions of the biomass and generating methane as a gas. This gas may now permeate into the well or it may become food for a range of aerobic heterotrophic bacteria that can oxidatively degrade methane with the release of some carbon dioxide. The presence of methane (as the principal component in natural gas well) does not necessarily mean that the water well is being impacted by a neighboring gas well, but it could mean that the well is generating its own complex microbial community that does include the methane producers.

Figure 9.54 illustrates gas production in two vertical water well column (WC) sections in which the formation methane gas bubbles (GB) has occurred and becomes entrapped in the thicker biocolloids. In the left-hand diagram, the redox front has moved into the well creating a focused floating but submerged biomass (FB) that acts as a plug in the water restricting the upwards movement of the GBs. As the biomass plug collapses and GBs now begin to move up the borehole they now may become entrapped within the FB to form a foam plug. In the right hand diagram, a video camera (VC) logging of the well calls for the VC to descend the borehole. As the camera disturbs the FB then GBs are released to stream up the borehole to the head space above.

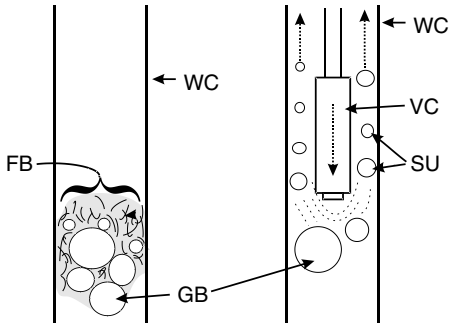

FIGURE 9.54 Illustration of the perching of gas bubbles in biofouled water wells.

9.55 HARMONIC PULSING ACTIVITIES IN PLUGGING BIOMASS

Slimes and biomass are thought of as being very simple and not involving any type of life cycle (it just grows and grows until there is no where else to grow and then it plugs). In reality the biofilms comprising the biomass do go through a number of stages during a growth cycle (a little bit like the rings on a tree). Figure 9.55 shows

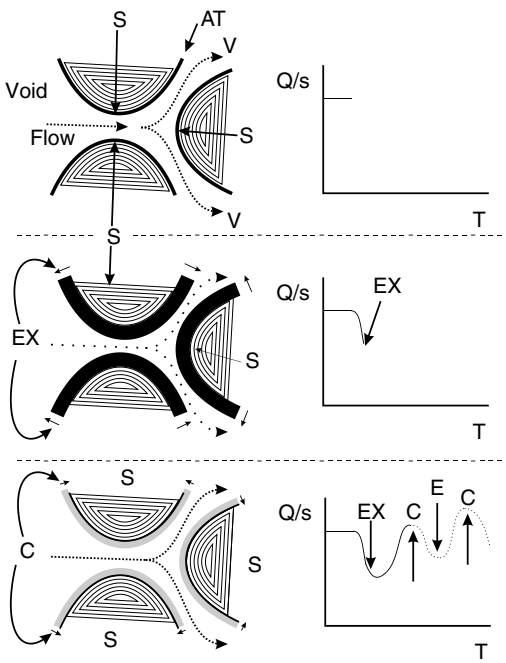

FIGURE 9.55 Biofouling (lines) in a porous medium (right side) can cause harmonic changes in the Q/s overtime as the biomass changes in volume and form.

the theoretical relationship between the pulsing growths of the biomass (as a conglomerate of biofilms) that is attached surfaces (S) within voids (V). In this figure, it is assumed that all of the water flow (arrows) is passing through these voids. In these figures, the porous medium (e.g., gravel) is shaded with the thickness of the attached biofilms being shown as a line which has a thickness reflecting the thickness of the growths.

In this figure, there are three separate diagrams reflecting the three major stages during the growth of the biofilms within the biomass. Each diagram has a graph to the right side that shows the specific capacity (Q/s) as the *y*-axis overtime as the *x*-axis. As the biomass grows then it will intercede with the water flow. The upper diagram shows the noninfested porous media with the specific capacity remaining at 100% the original overtime. When the ability of the water to pass through the porous media in the formation is impacted by the status of the biomass then the Q/s will reflect the status of the well. In the middle diagram, there has been an EX in the biomass which restricts water flow and reduces the Q/s. After EX then the biofilms commonly go through a compression (C). Here the volume inside the biomass now shrinks allowing greater flow through the voids and an improvement in the Q/s. This shifting in biomass volume associated with EX and contraction is further affected by periods when the volume remains stable. Thus in an actively growing biomass around a well, it may be possible to observed periods of EX, contraction, and stability. This would clearly have a direct impact on Q/s given it harmonic cyclic signature that may also be used as a signal of impending plugging.

9.56 METAL BIOACCUMULATION AROUND ACTIVE WATER WELLS

Water wells naturally have biological filters positioned in, and around, the borehole that can interact in positive and negative manner with the chemistry of the groundwater. One common event is that the biomass will accumulate metals far beyond the capacity of the microorganisms to use these metals. Under very OX conditions, it is commonly the ferric forms of iron that are accumulating but as you go through the biomass towards the RD side then other metals become preferentially accumulated. Essentially the biomass in functioning as a living "slime" chromatograph separating out these various metals into subgroups that are then deposited along the redox gradient around the well.

Figure 9.56 illustrates these effects showing the BM forming around and inside of a VEW. These conditions allow the biomass to accumulate different metallic cations under different redox conditions at different sites. In this figure, the diagram shows five regions around the well moving from highly OX inside the borehole to highly RD deeper out in the aquifer farthest from the well vertically shaded zones represent ORP values ranging from $+150$ to $+20$ (A), $+19$ to -5 (B), -6 to -50 (C), -51 to -150 (D), and more RD than -150 mV (E). Each of these sites causes the accumulation of different cations within these five ORP regions. Note that each of these cations is shown grouped laterally at the sites within the ORP gradient where these chemicals would be most bioaccumulated. Turning on water wells that

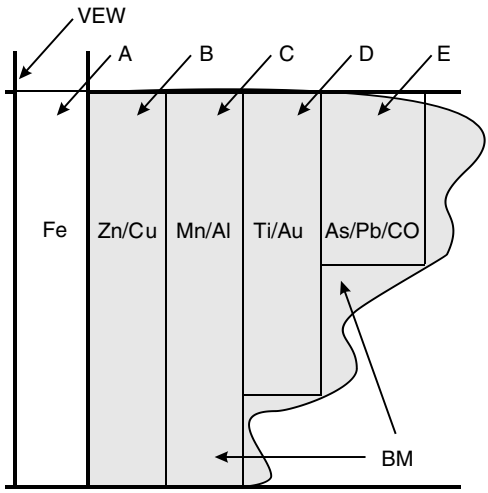

FIGURE 9.56 Vertical section of biofouled well illustrating the bioaccumulation of metallic cations along the redox (oxidation–reduction potential) gradient. Critical metallic ions shown by letters on the shaded chart are as follows.

have been shut down for a period of time (e.g., 7 days or longer) are more likely to release significant levels of the bioaccumulated metals into the product flowing water. These concentrations are likely to be much higher if the biomass is more matured and now sloughing (a common occurrence later on in the growth). Old biomass tends to finally stabilize when it has formed into a metal-rich concretion.

9.57 IMPACT OF TREATMENTS ON THE SEQUENTIAL RELEASES OF METALS FROM BIOMASS

Water wells being impacted by biomass are likely to develop a form of growth that will move back into the formation and form a continuous structure around the well. This would be composed of some combination of slimes, encrustations, and other forms of connected growth. Here this biomass would function in part as a biological "chromatograph" that would sequentially accumulate the various metal cations at various points along that growth. The next effect of this is that the biomass becomes not homogenous (being all of the same chemistries) but heterogeneous (with the various microorganisms and cations occurring at different locations). When treating water wells that are biofouled then it can be expected that there will be sequential effects with the biomass closest to the well (if that is the site for the treatment) being impacted first followed by regions of the biomass moving further and further away from the well.

Figure 9.57 illustrates the sequential impacts of a regenerative treatment (RT) through four diagrams (1, 2, 3, and 4) show the impact of four levels of regeneration impacts as the treatment moves further out from the well during treatment and

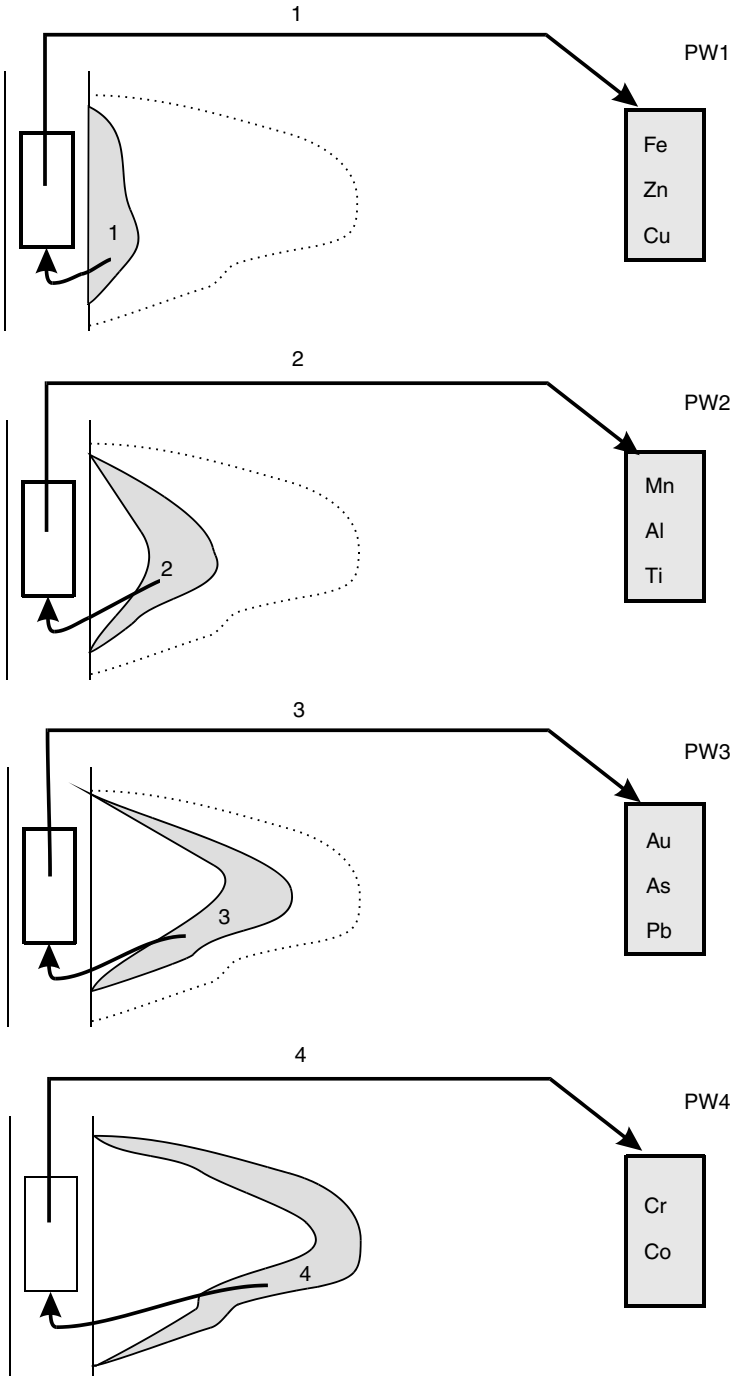

FIGURE 9.57 Chronological presentation of well regeneration effects on the sequential releases of metallic cations from a severely biofouled well.

recoveries. This set of diagrams shows diagrammatic vertical water well with a pump installed with shaded zones around the well showing the impact sites for four different phases of treatment and recovery. Each of these four phases shows a curved arrow leading from the treatment site in the biomass towards the base of the pump. A second arrow now extends from the tope of the pump and is angled to the right above the well. This is the pumped water (PW) that would be affected by the metal cations being removed in the water from the disrupted and dispersing biomass. During each of these four periods of recovery (one to four), there is a difference in the metallic cations dominating in the water. These cations are listed to the middle right of the appropriate graph. Essentially what has happened has been that the biomass was disrupted and dispersed in a manner that led to the sequential releases of different cations with each phase of the pumping. It should be remembered that when a biomass finally reaches a growth saturation point then these metallic elements may be sloughed in a continuous manner. This would be a reflection of severe biofouling and would indicate that the well should be regenerated with the removal of the bulk of the biomass including these accumulates. On such occasions, these bioaccumulates may render the recovered biomass particulates as hazardous waste.

9.58 RISKS FROM APPLYING PHOSPHORUS COMPOUNDS AS A TREATMENT STRATEGY FOR BIOFOULED WATER WELLS

Phosphorus (P) is, to microorganisms, one of the most essential elements for growth as indeed it is for all other living organisms. The difference between microbes and other organisms is that many of the microorganisms love to hoard surplus phosphorus mostly as polyphosphates (PP). Figure 9.58 shows three different conditions under which phosphorus has been added to a well as a part of the treatment. In the biomass (BM) around water wells, surplus phosphorus will be accumulated. When phosphorus is applied as acids or polyphosphates (PP) it can form an important part of a treatment process. This diagram shows the fate of applied phosphorus (AP) as a part of the treatment strategy. Three conditions are displayed focusing around the well around which BM is shown to the right-hand side of the well. The top diagram (1) shows applied phosphorus being added but without any recovery of the applied phosphorus. Here all of the AP is taken up into the biomass as polyphosphate (PP). This would mean that the treatment applied phosphorus could now become used by the biomass for additional growth and activities.

In the middle diagram (2), some of the treatment AP is recovered by the posttreatment dispersion and recovery of biomass removed from the well during the treatment. Recovery of the treatment AP means that the biomass would not benefit from any accumulation of this applied phosphorus. The better condition is shown in the lower diagram (3) where not only is all of the AP recovered but also that some of the PP that was resident in the biomass is also recovered. Under these conditions, then the amount of phosphorus recovered during and after the treatment would exceed the amount of applied phosphorus during the treatment. This now becomes

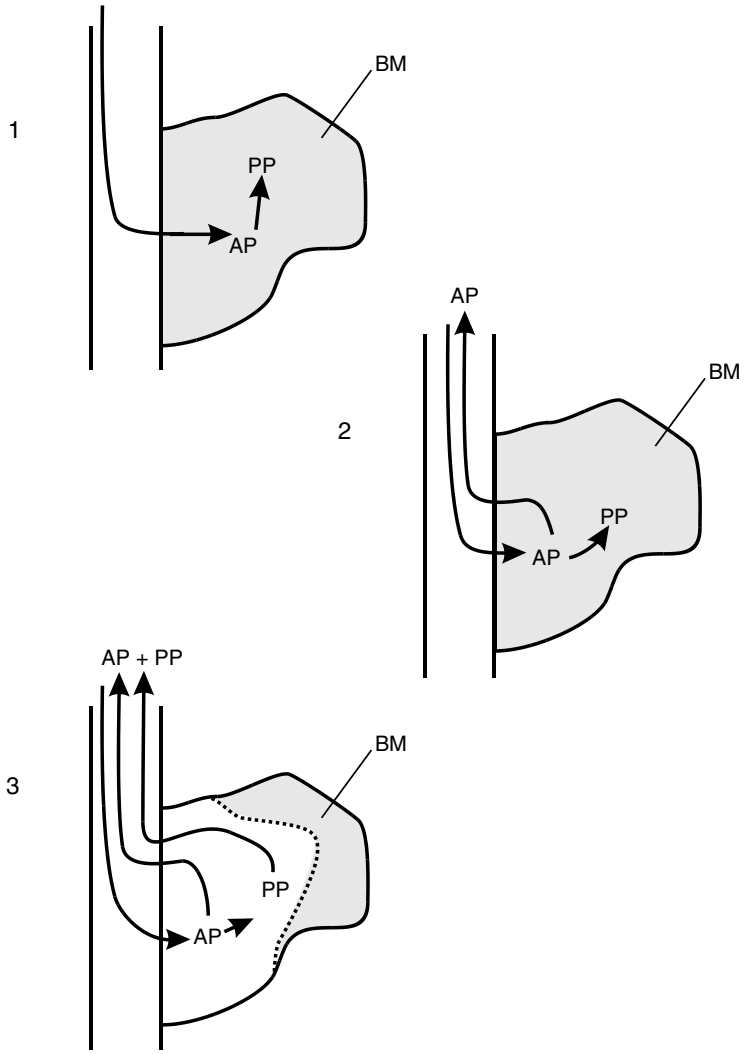

FIGURE 9.58 Diagrammatic presentation of the manner in which phosphorus, when used in well treatments, can move, be retained, or become assimilated and released.

a preferred strategy because this would now begin to deplete the amount of available phosphorus available for growth and activities within the biofouling biomass.

Applied phosphorus treatments are capable of breaking down biomass and opening up conduits around the well to give improved Q/s. As a minimal environmental control of the potential impact (preventing subsequent growth in the surviving biomass on the AP), then there must be a legislated total removal of all of the AP applied. Failure to achieve this would mean that some of the AP would now be converted to PP and utilized by the biomass and stimulate biofouling (plugging) of the well.

9.59 MATURATION OF BIOFOULING WATER WELL: IMPACTS ON SPECIFIC CAPACITY, AND BACTERIAL ACTIVITY

Figure 9.59 includes four graphs each with time as the *x*-axis and the percentage specific capacity (continuous line, Q/s) and bacterial activity as populations (dashed line, BA; p.a.c./mL) as the two parameters on the *y*-axis.

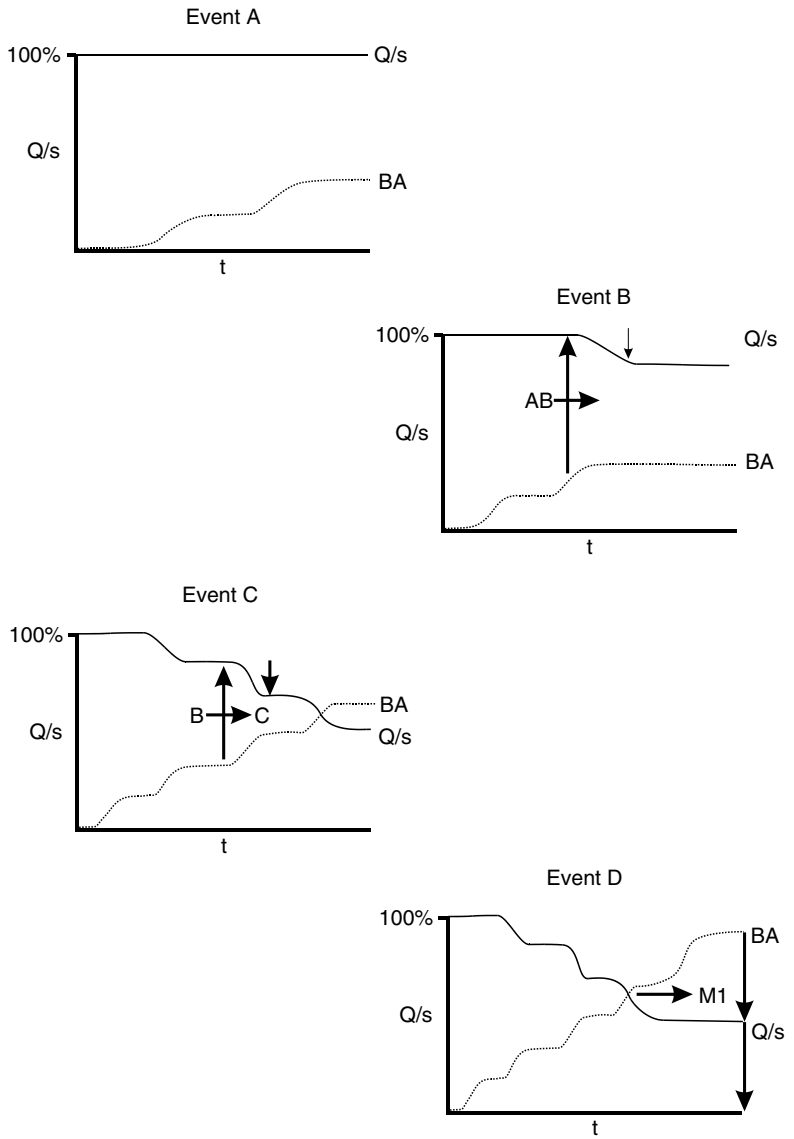

FIGURE 9.59 Graph showing the four typical stages in the decline in the specific capacity and increases in bacterial activity in a water well.

Four possible events are shown as A, B, C, and D which illustrate the changes that would occur overtime for the water well that was subjected to various forms of continuous levels of biofouling over the different periods of time. These events graphs are layered one over the other with the *x*-axis (time changing). Event A occurs as the first period in which the Q/s remains at 100%. Some time into this period of stable specific capacities there is an increase in bacterial activities. These activities are detected through shortening time lapses using the BART testers. This event phase (A) concludes when the Q/s begins to decline (vertical arrow on event B). Usually, this event is recognized by a significant (commonly 5%–20%) drop in the Q/s that accompanied by further increases in detectable bacterial activity. This commonly ends with a flat line where the Q/s has declined along with the population activity increase. This period of stability (event B) ends when there is a further collapse in the Q/s and increase in bacterial activity. Event C follows a set of stepwise drops in Q/s and rises in the population activity. Within each of these steps, the Q/s again becomes stable again and the pulses of increased bacterial activity follow the same pattern. This stepwise reduction in the Q/s continues until there is a sudden dramatic drop in Q/s to $<10\%$ of the original Q/s (event D). At this time, trauma created in the now stressed bacterial communities growing in the plugging biomass can cause erratic releases of bacteria along with the releases of bioaccumulated metallic cations (e.g., ferric-iron) into the produced water. During event C as the Q/s drops then the ability to effectively regenerate the well diminishes rapidly and it will be a steep climb to get any significant recovery in specific capacity.

9.60 CONTROL STRATEGIES FOR REMOVAL OF BIOMASS

In establishing a method to control biofouling in a water well, video camera logging will often show growths coming out from the slots into the water column and then further growths occurring within these slots and perforations. Figure 9.60 is an illustration of these sites (numbered) that are targets in regenerative treatments (RT) that become the first and the most obvious targets for treatment. The first target (1) has to be biomass (often encrusted and slimed) growing out into the water column of the well. Here the best initial treatment is physical (e.g., brushing, sonication, or jetting). This forms the simplest manner for dispersing the "outer edge" of the biomass (B). Next target (2) is the growths occurring around the slots (S), perforations or in the fractures. These growths act to restrict water flows (F) into the borehole. Once the well has been opened up then the next target (3) becomes the biomass entrenched in the voids of the porous formation material around the well. Here, gravel packs (G) that have larger voids are easier to successfully treat while sands and finer material become more and more difficult to attack from the direction of the borehole. In some cases, the application of treatments through satellite wells can also allow the successful treatment of deeper set biomass.

Knowing the location of the biomass cannot be necessarily achieved by using VC logging but it may also involve application of ZIP and, where possible, satellite wells and coring. This then allows not just the distances to be calculated that biomass

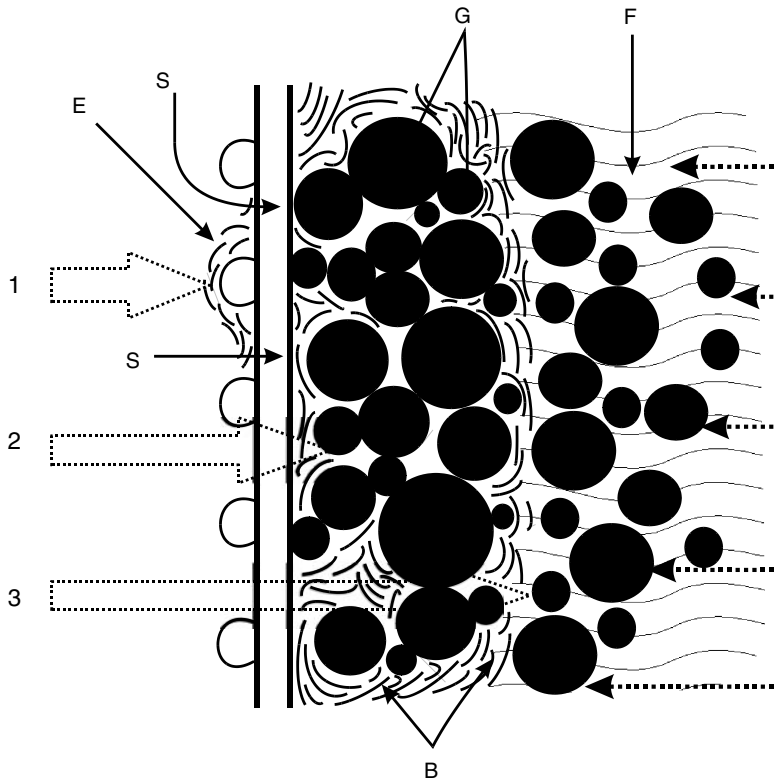

FIGURE 9.60 Graphical presentation of the target sites for well regeneration within the water column.

has extended outwards around the well but also the levels of activity and the nature of the bacterial communities that are present. It is not uncommon for such biomass extensions to be out to 15 ft (5 m) from the well which means that whole region needs to be treated in order to effectively regenerate the well.

9.61 COMPARISON OF WELL REGENERATION WITH PREVENTATIVE MAINTENANCE (SERVICING)

There is a major difference in the application of regeneration (R) and a preventative maintenance (PM) servicing treatment of water wells. Here regeneration is considered to involve a more radical treatment that would severely impact the guilty biomass that has already had a very significant impact of the production capability of the well. Effectively undertaken regeneration should significantly return the well towards its original performance but there are no guarantees that this would always be achieved. Once the well has been effectively regenerated then it is easy for the clients to be lulled by the flow into a state of complacency believing possibly that

the treatment is a "cure all" which will last forever. This is not the case. Biofouling will reemerge even if the water wells have been sterilized. It is therefore essential to consider a radical regeneration that has to be followed by a PM servicing of the well that would prevent the biofouling from becoming a major impact on the wells production. Ideally, PM should be designed in as a part of the design and management of the well before it is even installed and developed.

Figure 9.61 shows two graphs both with time as the *x*-axis and Q/s (as a percentage of the original specific capacity) is shown. In the upper graph indicates the effect of successful regeneration and the lower graph shows how repeated PM servicing treatments can be maintain the Q/s (continuous line) or through BART testing (dotted line). Note that the right-hand *y*-axis shows the log population (p.a.c./mL) of bacteria. Treatment applications are shown by a vertical arrow descending to the line.

9.62 SHOCK, DISRUPT, AND DISPERSE PHASES OF WATER WELL REGENERATION

Sometimes it would be nice if you could just point a high-tech camera down at a well and it would show you where all of the biomass were growing around the well! Take another image after the treatment of the well and you would know from the position and form of the biomass (BM) that the treatment had worked or had failed.

Figure 9.62 provides a diagram in the form of four plan views looking straight down at a biofouling water well where the shading shows the position of the active biomass. There are four figures in this plan view looking down at the water well in which the initial extent of plugging (a) is shown by shaded scales indicating the intensity of the BM. Rehabilitation occurs in three phases. Shock (phase 1, b) shows the near borehole impact of the first treatment causing some localized killing of the microbial biomass but no impact further away form the borehole. Disrupt (c) now extends the treatment impact further away from the borehole though a combination of strong physical and chemical forces. This leaves the disrupted dead biomass perched within the formation. Disperse (d) is now applied as the final phase in which primarily physical forces are used to remove the dead biomass (this is to get rid of the potential feed stock for future growths down the hole) from the borehole itself or through satellite wells. Residual biomass (RBM) may be left even after effective treatment.

9.63 EFFECTS OF DISTORTIONS IN LOCATION OF BIOMASS ON EFFICIENCY OF TREATMENTS

A major challenge in the treatment/regeneration of biofouled water wells occurs because the biomass (B) is unevenly distributed in all Lateral directions around the well (W) borehole. In the practice of treatment such wells where the biomass has a focus of activity to one side of the well (generally upstream of the well) then there is

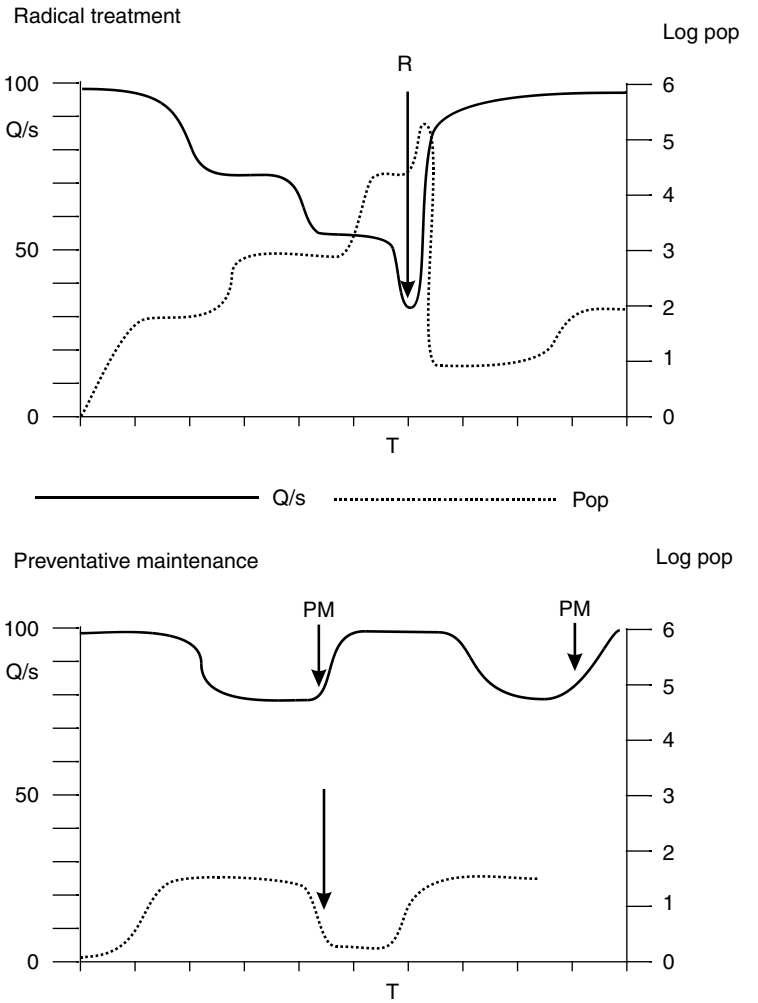

FIGURE 9.61 Graphical summary of the effects of regeneration and preventative maintenance servicing when applied to plugging water wells.

a real risk of the treatment fluids moving away from the well in the downstream groundwater flows.

Figure 9.63 shows plan views of water well (W) surrounded by biomass (shaded region) with groundwater flow shown as arrows. View one shows the well surrounded completely by biomass with the treatment being all down the borehole (open arrows, T) and being contained at least initially by the biomass. Here the biomass (B) resembles looking at an umbrella interposed between the well and the upstream groundwater flow. Here treatment is still shown being applied down the borehole but there is now a significant loss of treatment chemical into the downstream groundwater flow. This clearly would weaken the ability to effectively treat the well.

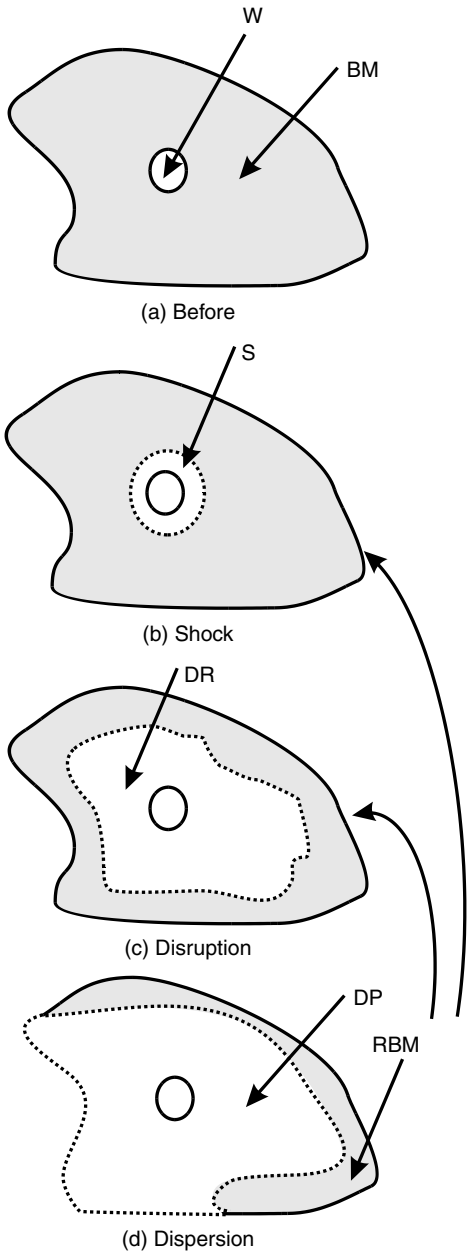

FIGURE 9.62 Plan view of the impact zones created by regenerative treatment of the well. Biomass is shown as the shaded zones with the clear (treated) zone around the circle (well) indicates the region cleared of biomass activities.

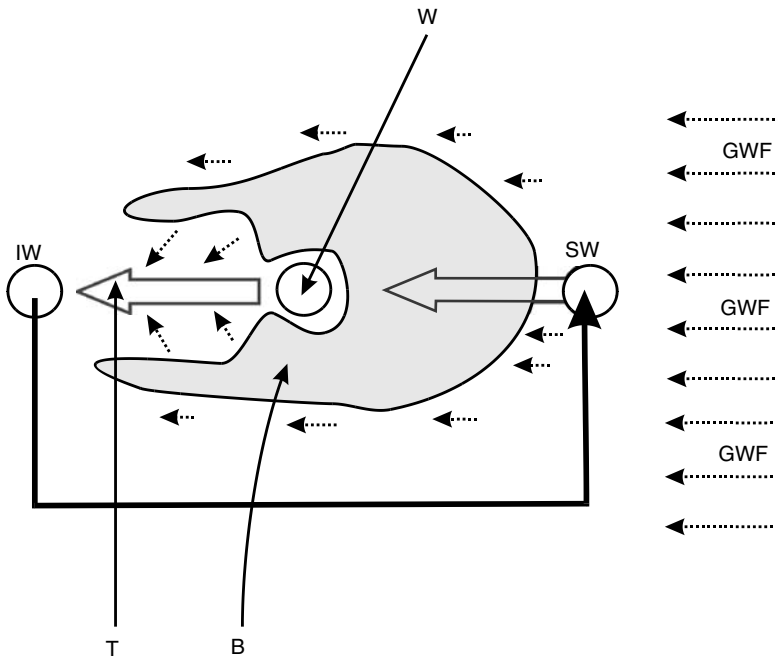

FIGURE 9.63 Common treatment application procedures for the dispersion of biomass growing only in the upstream groundwater flows.

Where this is likely to happen then there would need to be a compensating approach. This can involve a satellite well to intercept the groundwater flow carrying the treatment chemicals away from the well-being treated. These intercepted treatment chemicals can now be pumped upstream of the well-being treated where it would be injected upstream of the biomass impacting the targeted well. This then provides a method for recycling the treatment chemicals to minimize losses and potential downstream environmental damage to the aquifer.

9.64 TREATMENT OF FRACTURED HARD ROCK WELLS

Biofouling in fractured rock has a very different set of challenges to that in porous media. Primarily, the surface areas in a fractured rock are smaller relative to the volume of the rock through which the fracture passes. Secondarily the throat sizes (diameter of the narrowest points through which the groundwater passes would also be much bigger in fractured rock than it is in porous media or pack material). These two differences between porous media and fractured rock mean that the location of the biomass would be much different. For the fractured rock, the biomass would tend to be growing on the surfaces of the fractures and growing for a longer period of time before the throats would become plugged with the growth and the specific capacity of the well negatively impacted.

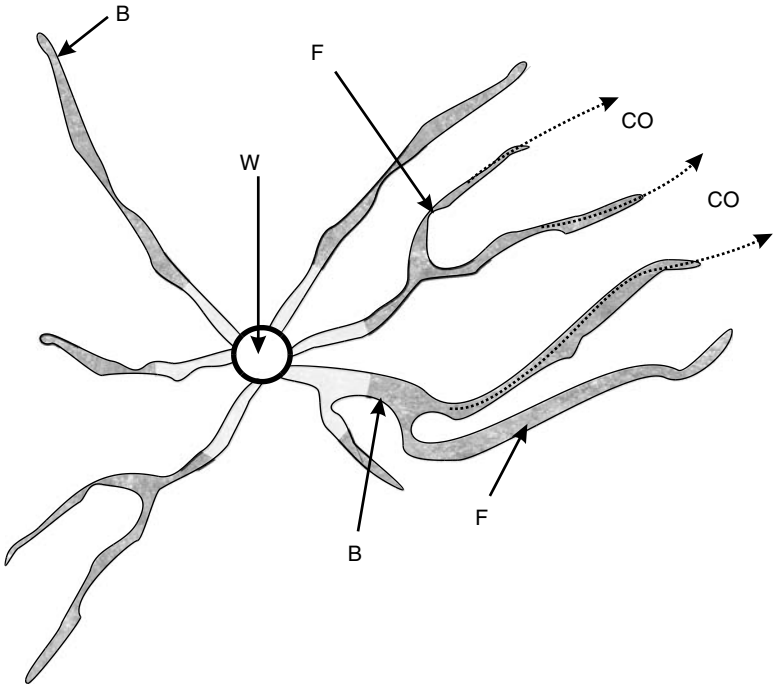

FIGURE 9.64 Schematic for the application of carbon dioxide to regenerate water wells in consolidated (hard) rock formations.

Figure 9.64 shows a plan view looking down into a section of a fractured rock well. These wells present challenges to regeneration because frequently these fractures (F) move away from the water well (W) in randomized directions. Much of the significant plugging biomass (B) will be growing within these fractures. Biomasses growing in these circumstances are growing on hard rock surfaces and may not so sensitive to the chemical treatment. Under these circumstances, the biomass may be more vulnerable to the injection of carbon dioxide (CO) that can drop the pH outside the range where the biomass can function in an attached manner while the lowered the temperature can cause the biomass to freeze and detach making it easier to disperse. Where freezing occurs then the whole biomass would be disrupted and detached from the surfaces allowing effective dispersal.

9.65 IMPACT OF TREATMENT ON BIOMASS INVOLVED IN BIOFOULING

At the scale of the encrustation or biofilm, Figure 9.65 illustrates the effects of a generalized effective treatment in which the biofouled surfaces (S) become cleaner. There are three diagrams indicating the staged effectiveness of a treatment. Specifically each diagram shows the effectiveness of an effective penetrant-dispersant (PD, biostatic detergent) on encrusted form of biomass (BM) growing in a well.

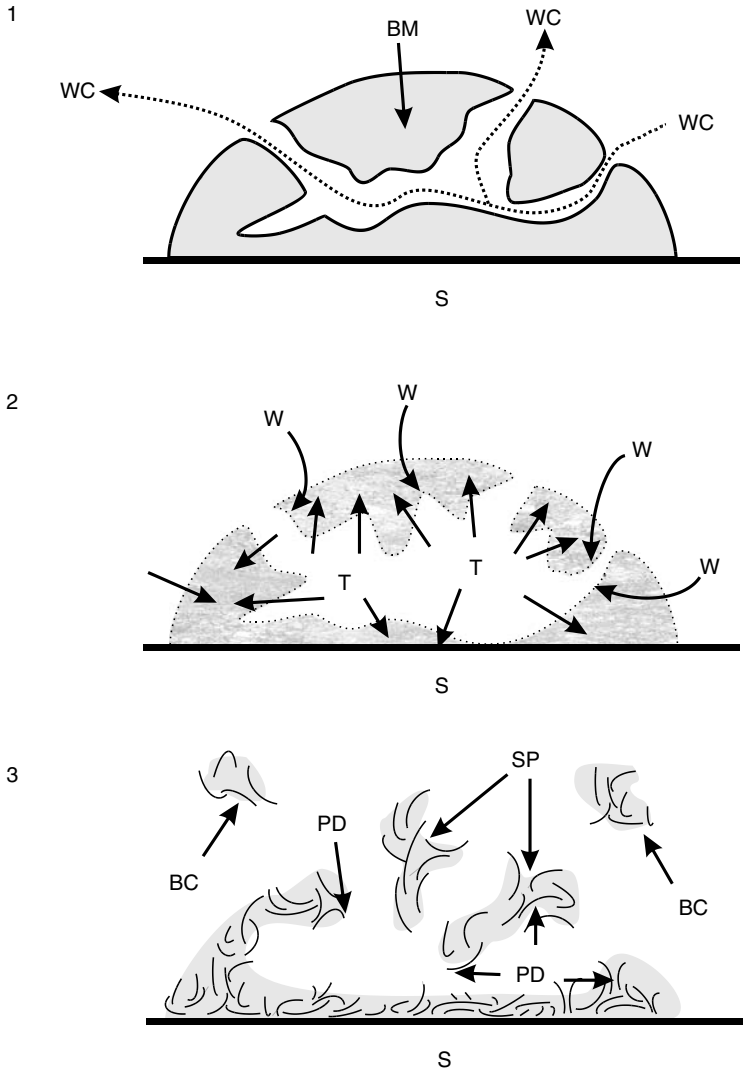

FIGURE 9.65 Graphic presentation of the shock, disrupt, and disperse phases in well regeneration with the use of an effective detergent.

In the top diagram, there is shown a vertical cross section of the growth showing the water conduits (WC) through which the chemical can enter the biomass. This biomass has not been treated and this represents a "before" state. Treatment (T) has been started in the middle diagram showing that the treatment chemicals (arrows) have entered the biomass through the water conduits as well as directly through the walls (W). These chemical–biomass interactions now cause the primary structural collapse of the biomass. The product at this stage in the treatment is a disrupted biomass in which much more of the growth structures are

now exposed. In the lower diagram, the end point of the treatment phase is reached showing that the biomass is now fragmented particles with some particles now suspended in biocolloids (BC). It is now easier to disperse these shattered particles (SP) and BCs and leave the surfaces relatively pristine. It should be noted that even the BCs during dispersion would also be collapsing as a result of the action of the biostatic-detergent.

9.66 FACTORS AFFECTING SUSTAINABILITY OF WATER WELLS

Sustainability has become a "catch" phrase for environmentally responsible operations of virtually anything. The "modus operandi" is to make the system last as long as possible rather than to be discarded as a disposable (and replaceable) item. Water wells fit right into that category and the general practice is simply to "use it until you lose it!" One problem historically with water wells is that the roles of the biomass zones (BZ) have been underplayed or simply ignored, while the operation of the water well (WW) becomes an accounting procedure that defies the need for even basic PM and servicing le alone radical regenerative treatments. As groundwater becomes more precious then it can be expected that sustainable water wells will become the norm. As groundwater becomes more precious then it can be expected that sustainable water wells will become the norm. This would mean that maintenance budgets would have to be larger and acknowledge the essential nature of this activity. Through effective management taking into account the nature of the natural filters around the wells then long-term sustainability would be achieved.

Figure 9.66 shows the negative impacts to making the well sustainable as closed arrows and positive impacts are shown as open arrows. The key for some of the major factors listed in this figure include: organic materials (O) entering the wells (satellite well, SW; water well, WW); phosphatic nutrients (P) that are indigenous (natural phosphorus, NP) and used in treatments (applied); indigenous microflora (IM), and contaminating microflora (CM); iron in groundwater (FE), as the production begins then so the redox front (RF) will move. It will become more challenging when it moves closer to the well since there would then be a greater potential for the voids to become totally plugged whether regenerative treatments (RT) are applied down hole (TW), or from the satellite well (TS) has to disrupt the biomass (dotted ellipsoid) adequately enough to break open the plugging and reduce the risks of corrosion. Preventative maintenance servicing of a biofouling well usually has a more restricted impact. Sustainability would mean controlling the plugging associated with the biofouling IM and the CM.

9.67 EFFECT OF GROWTH OF VARIOUS MICROBIAL COMMUNITIES ON FUNCTIONS OF WATER WELLS

Biofouling water wells often cross the whole spectrum from oxidative to reductive conditions through a redox front. This results in many different microbiological communities all finding their own niche in the "biowall" that forms and grows around

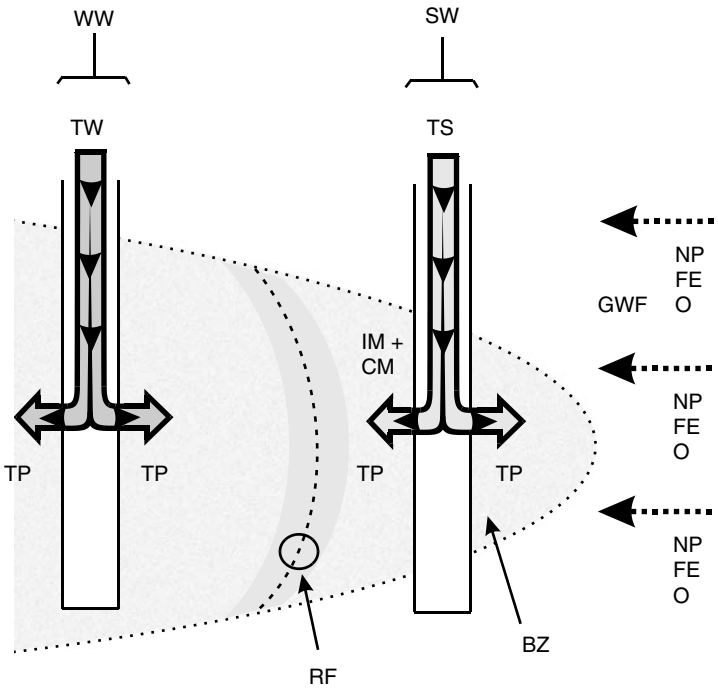

FIGURE 9.66 Diagrammatic presentation of the factors affecting sustainability of water wells.

the water well (WW). It is most definitely not accurate to consider some single species or even group of species as causing such an infestation but it is rather a sequential series of different microbial communities forming virtual cylinders of living slime around the well with the outermost being in the most reductive conditions.

Figure 9.67 shows the vertical section of the water well (WW) with the static water line (SWL) below grade (G). Here there are a series of partial ellipsoids representing the position of various microbial communities that can be associated with the biomass biofouling the well. Each microbial group is shown as having a different shade and are defined by indicating arrows as: IRB, iron-related bacteria; SLYM, slime-forming bacteria; HAB, heterotrophic bacteria; DN, denitrifying bacteria; SRB-(BT), SRB associated with aerobic bacteria; SRB-(BB), SRB growing anaerobically deeper out in the RD porous media; and fungi (F) that grow mainly around the SWL but will release mold spores (MS) that can enter into the well. Things to remember is that the fungi do produce such copious numbers of spores that would be carried by the groundwater flow (GWF). These spores would be relatively dormant but would still be detected in microbiological tests for fungi (molds). A high presence of fungal (mold) counts in the groundwater tested may simply mean that there are a lot of MS traveling through the waters from below the static water level. If fungi are active in the regions immediately around the well then this would mean that there is a high probability that the groundwater is only semi-saturating the formations immediately above the productive sections of the borehole. This would most

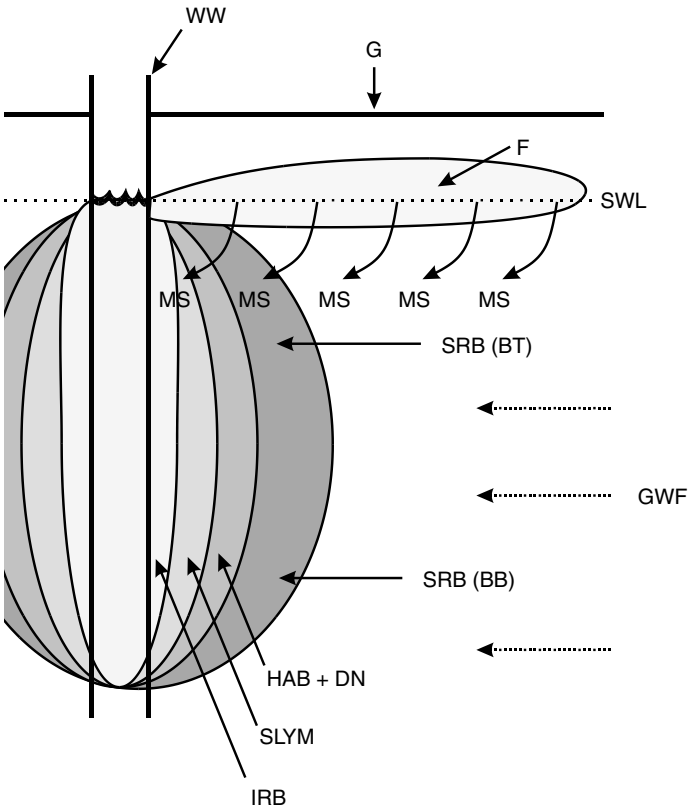

FIGURE 9.67 Locational projection of the sites of focused activity for the microorganisms commonly associated with water wells.

probably mean lower yields of water and heavier releases of MS particularly when the well was fist pumped.

9.68 SEQUENTIAL RISK ANALYSIS FOR TEST PUMPING OF WELLS

Risk diagram shows the various signals that can be observed in water wells during the initial critical stages of well development due to biofouling (Figure 9.68). Signals are listed to the left as droplets. Critical symptoms include increasing:

TC (cloudiness): this loss in clarity may be caused by increasing microbial populations in the water, silt coming into the well, or chemical reactions causing floating precipitates.

Elevating dissolved iron (DI): this DI might be caused by ferrous-iron entering the water which would be more RD (low or negative ORP values).

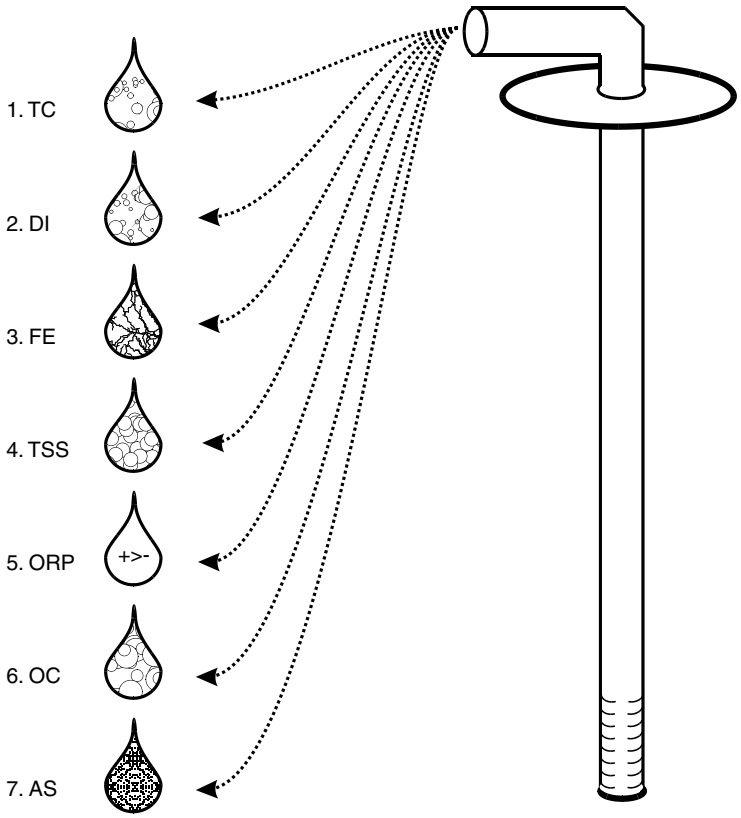

FIGURE 9.68 Risk diagram indicating critical signal activities generated by infesting bacteria that would indicate the compromise of the well.

Particulate ferric-iron (FE): when ferric rich biomass begins to slough when conditions are OX and the growths are maturing.

Total suspended solids (TSS): here there is most likely to be an increase in biocolloidal content associated with microbial activity in the water but it could also be associated with chemical precipitates and sloughing of the biomass.

Falling ORP values (ORP): this occurs during pumping the water moves from a positive millivolt value to a negative ORP which means the water being pumped is more reductive zone possibly the other side of the redox front.

Increasing organic carbon (OC): this occurs when there is a greater amount of sloughing from the biomass and/or greater biocolloidal contents. It is recommended that such tests always employ total organic carbon and not dissolved.

Increasing arsenic concentrations (AS): this occurs when the bioaccumulated arsenic historically bound up in the biomass finally is released through sloughing. Arsenic tends to be accumulated on the reductive side of the

slime chromatograph and so may appear erratically and then significantly the latter stages in the life of the well.

As biofouled water wells age, one additional factor would be increasing amounts of microbial activity that would be reflected in more active microbial populations (by greater than two orders of magnitude) and much shorter time lapses when BART testing is applied.

9.69 BIOFOULING OF RELIEF WELLS IN EARTH-FILLED DAM STRUCTURES USED TO CREATE WATER RESERVOIRS

Around the world the, retention of pooling water into reservoirs is sometimes achieved by creating earth-filled dams (EFDs) across the natural flow paths of the water. This causes the water to back up behind the dam. These dams are particularly vulnerable to erosion if the water begins to spill over the top of the dam. To correct and prevent this risk, it is common for such dams to be equipped with downstream relief wells (RWs) that control the water level (WL) in the reservoir. As these levels go up so then there is a greater pressure on the RWs to divert water from the dam to a downstream conduit. These RWs, like other types of water wells, are prone to plugging only here the principal symptom would be failure to relieve head pressures in the reservoir and associated risks of head erosion along the top of the dam.

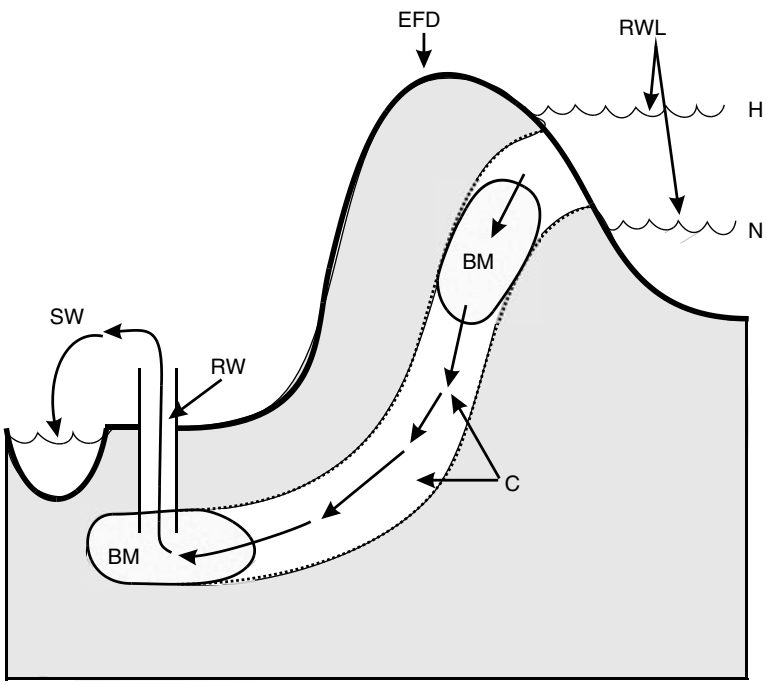

FIGURE 9.69 Section diagram of the control of biofouling challenges in relief wells.

Figure 9.69 provides an illustration of the cross sections (vertical) of an EFD showing the reservoir water level as oscillating between high (H) and normal (N) levels. When the WL is elevating and putting the dam at a greater erosive risk then conduits (C) through the face of the dam which have a high porosity (e.g., gravel pack) move the surplus water (SW) downwards (arrows). This down flowing water moving through the conduits is captured by RWs from which the water is then safely discharged.

Redox fronts are likely to form within the dam and support BM (shaded ellipsoids). Because the water percolating from the dam into the drainage conduits is more likely to be oxidative than biomass-initiated plugging is likely to occur across the conduit draining the water from the dam and also around the RW themselves. Common practice is for a string of RWs to be placed along the downstream foot of the dam and control the WLs within the dam. Challenges therefore relate to getting the regenerative treatments (RT) effectively to the sites of plugging (biofouling) in the dam.

9.70 BIOFOULING OF HORIZONTAL WELLS

There is a common practice to drill wells horizontally which gives the well a greater access to a lateral seam of water in the geological strata. These wells may be primarily employed as extraction wells (for water) or as injection wells (for the disposal of waste fluids).

Figure 9.70 shows the side view of a section of horizontal well (HW) shows the theoretical position of the redox front during injection and extraction with the effect that this is likely to have on the location of the biomass (BM) in which the location of the biomass is referenced as BMI for injection wells and BME for extraction wells. This biomass would reflect the positioning of the redox front (RF) for extraction wells (RFE) and injection wells (RFI). If injection involves the admission of electron acceptors such as oxygen, nitrates or sulfates, then the redox front may be pronounced during that phase. For the extraction phase, there is likely to be fewer electron acceptors and a possible abundance of organic carbon (as potential nutrients). This may cause conditions to be come more RD with a greater probability of gas formation under these conditions.

Regenerative treatments of HWs is more challenging than vertical wells since there is a greater potential for the biomass to be evenly distributed along the whole length of the well. This would mean that when treatments essentially punched holes in the biomass then there would be a greater possibility that the treatment chemicals would bleed away from the well and become less effective.

9.71 IMPACT OF FLOODING ON PRODUCING WATER WELLS

Flooding of a water well field creates a number of challenges to the functional operation of the well. Where the flooding is driven by the movement of flood waters over the ground then there are a number of challenges to the operating wells that are

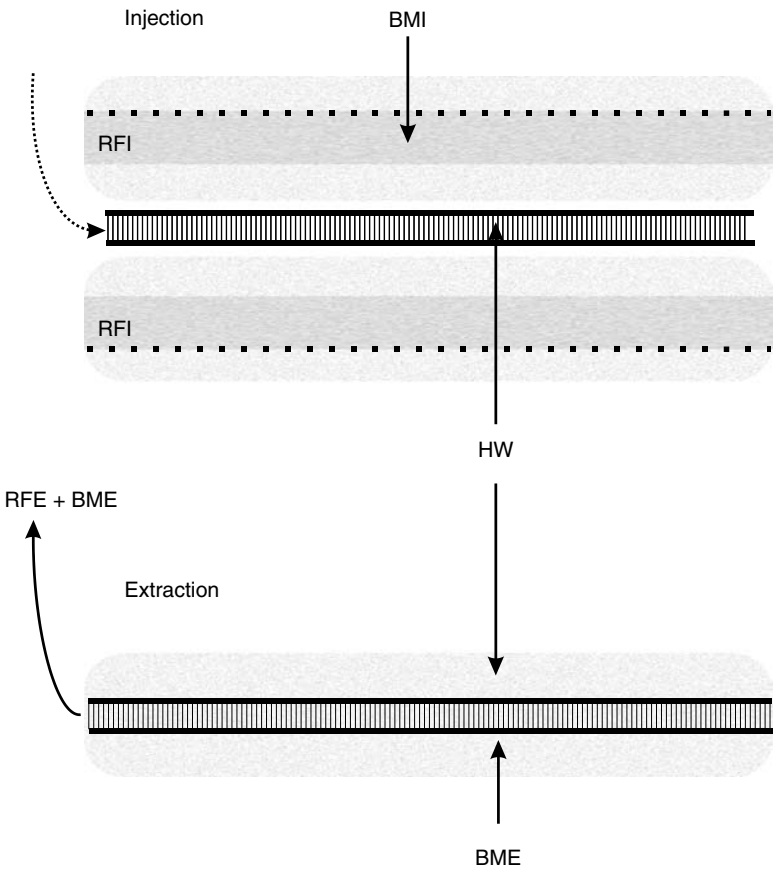

FIGURE 9.70 Graphic depicting the effect of the location of the biomass in horizontal wells set in either injection or extraction modes.

now going to be inundated with these surface waters coming over grade. Commonly such waters would be saturated or near saturated with oxygen which means the entry of that water into a well will carry with it oxidative conditions. Additionally, the nature of the flow if these flooding surface waters means that the turbulence and erosive flows will pick up and carry a microbiological burden associated with the soils and any solid or liquid masses (such as manure and compost) over which the surface waters flow. These also have significant potential impacts on the water wells. Potentially those influences can be summarized as surface waters having a direct influence on the groundwaters particularly in the wells water column.

Figure 9.71 shows a vertical sectional diagram of extraction well (W) are shown including the static water level (SWL), pump (P), and a screen (S). Upper diagram (1) shows the well in normal function with the static water level below grade. Flooded impact state is shown as the middle diagram (2) where the water well is now being challenged by a sudden flood of water (SFW) over the surface. Here arrows show

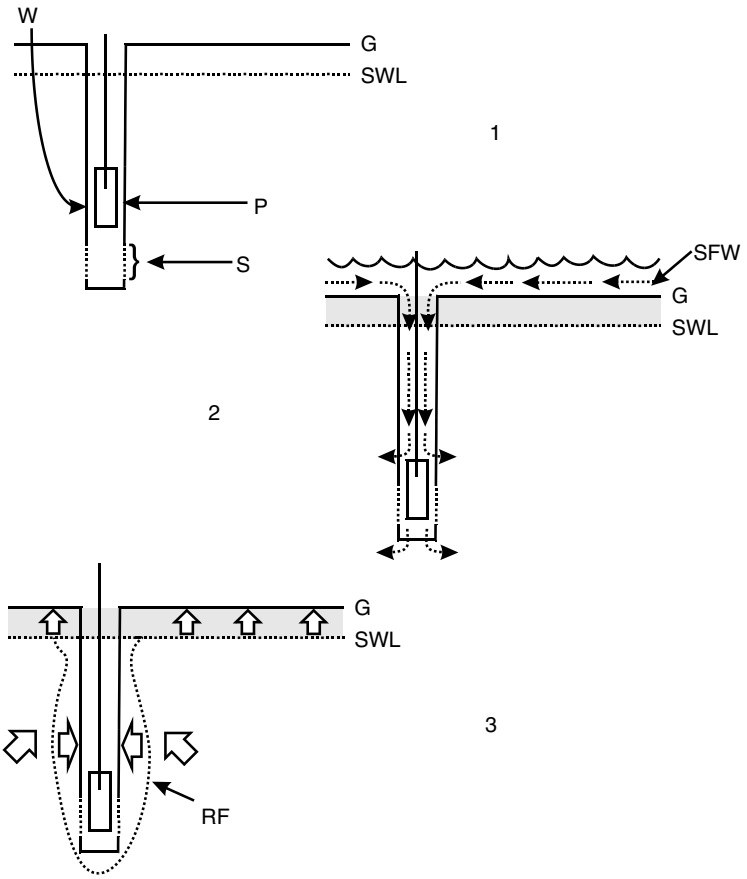

FIGURE 9.71 Schematic of the manner in which flooding can impact on water wells.

the movement of the flood water into, and around, the well. In such cases of catastrophic surface flooding, the formation material above the static water line would still contain gases (air) and would prevent some of the permeation of the flood water into the grade. In the lower diagram (3), this now illustrates what would happen if the static water level came up to the grade but did not pond on the surface. Here, arrows show the pathway that some of this water would move towards the well (open arrows) causing a greater risk of creating a direct influence on the redox front (RF, dashed lines).

Impacts of flooding on these water wells is therefore a combination of stress to the natural biomass active within the well (basically with the redox front being commonly pushed away from the well by the oxygen rich flood waters), and the indigenous microorganisms being carried within the flood waters now impacting downhole with the natural biomass. Clearly, there would no be competition and instability within the water wells until the flood waters recede and the redox front reestablishes.

9.72 DIRECT AND INDIRECT IMPACTS OF WATER WELLS FLOODED BY SURFACE WATERS

Flooding surface waters can impact a water well field in a number of ways. These are summarized in Figure 9.72 through a schematic of the various manners in which surface water can move into (continuous line arrows) and out of (dashed arrows) impacted extraction water wells. A single water well (upper left) is shown in vertical section with the grade level (G), the pumping groundwater (PG), as well as with the normal static water line (SWL) shown as a lateral dotted line. To the right of that is a water well under flood where the water level (WL) is now above the grade. Here there

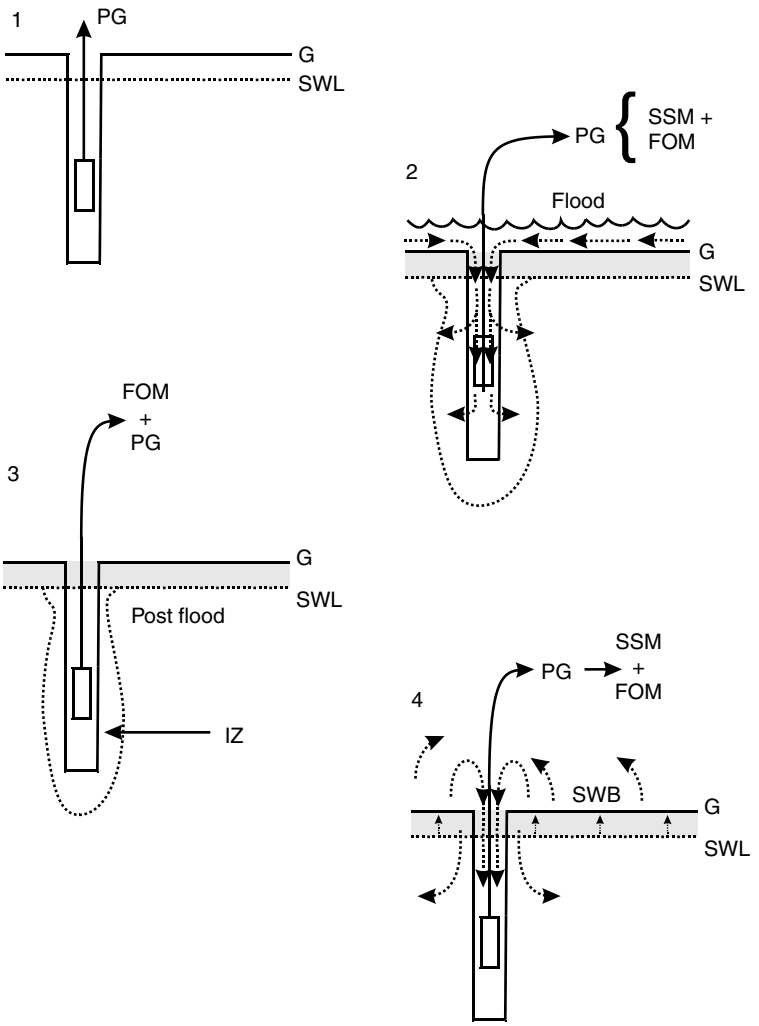

FIGURE 9.72 Illustration of the direct and indirect movement of flood water into productive extraction water wells.

is now the movement of flood-originated microbes (FOM) into the well both down the water column and around the casing CS. Additionally, the turbulence associated with the flood water movements would cause suspended solid material (SSM) to move up into the water and be carried downwards into the water wells environment. Within the soil, there would be a cyclic adjustment of the static water level up into the overburden soil in an erratic manner that could also cause releases that would enter the well.

In the lower two diagrams, the left-hand shows the impact of postflooding pumping of the well after the flood waters have receded. Two vertical sections are shown with the upper section showing the control (normally functioning well prior to flooding) to the left and a well suffering from catastrophic surface flooding. Here the pumped groundwater (PG) would now contain some of the microorganisms originating from the flood waters (FOM) as well as destabilized elements of the biomass that was now in a postflood stress mode. Eventually, it could be expected that the biomass would become stable but still contain some of the species that had adapted from the flood waters. Lower right diagram now shows a condition where there is not a direct surface flood over the well field but the excess of water causes the static water level to move up to grade and above. In these conditions, then there would be expected to be suspended material mainly from the soil that would be lifted up and then deposited down into the well environment. Under these conditions, then the static water table moves to the grade surface causing increased movement of soil and surface microbes (SSM) into the well environment and into the pumped water and associated surface water biofouling (SWB).

9.73 FLOOD IMPACT ON BIOFOULED WELL

Under conditions where flooding surface waters move out into water well fields and impacting the groundwater columns of those wells, then a major factor in the assessment of the impact would be the state of any biofouling in such impacted wells. Once biofouling in the form of vertical growths occurs inside the water column, in the slots, perforations, and fractures, as well as out in any porous media then the impact of the flood water downhole would be significantly affected by these growths which could then divert or even stopping flows.

Figure 9.73 is a vertical section based upon Figure 9.72 in which the major challenges to disinfecting (arrows) the different parts of the well environment are designated. Challenges vary with location. For example, there could be fouling at the static water level (SWL) that might generate a coating or sheen (S) on the water that could be enhanced through the presence of floods waters. For the water column itself then challenges to effective action of the disinfectant would be the lateral zones of intense biocolloidal activity (BC) that would neutralize the effectiveness of the disinfectant through bioaccumulative and degradative functions. Disinfection within the slots, perforations, or factures is likely to be limited by the ability of the agent to get into and through the thick slimes (SS) and encrustations (TSE) that would be present at these sites. Disinfectants that travel out into the porous formation media (PF) are likely to face the challenge of moving through biofouled voids (BV) in which these may be perched fines blocking water flow and disinfectant diffusion.

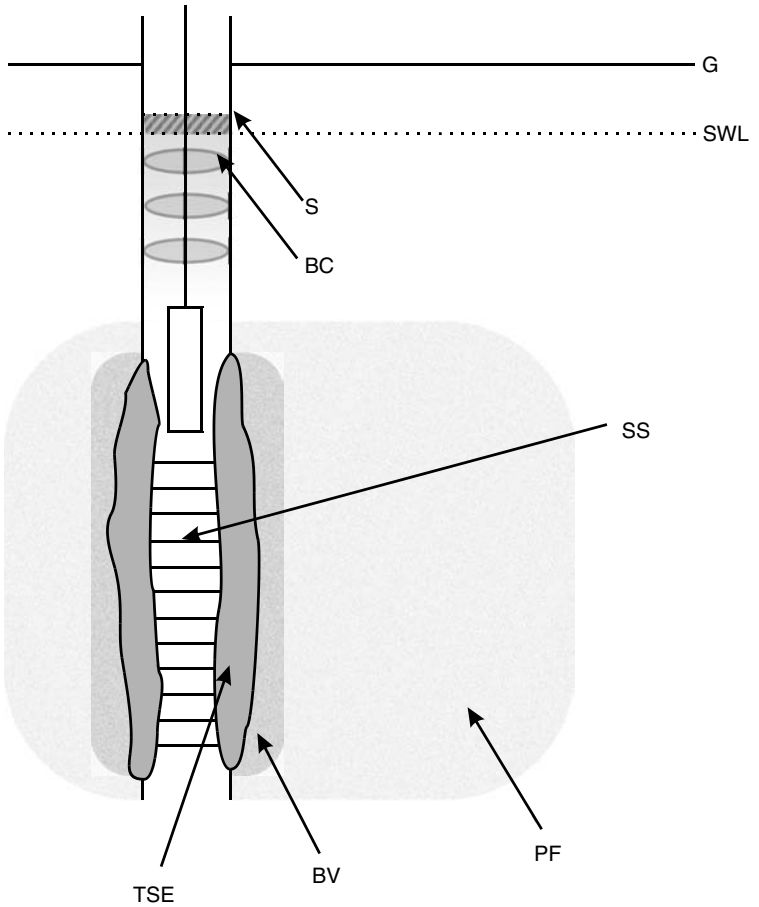

FIGURE 9.73 Functional diagram of the manner in which disinfection when applied to a flood-compromised water well impacts on the potential pathogens in the flood water.

9.74 PROTECTION OF WELLS FROM FLOODING

Water wells that are positioned at sites that could be subjected to surface flooding or impacted by the elevation of the groundwater to grade, both are vulnerable to surface water intruding into the well's environment. Figure 9.74 shows some of the methods that can be employed to reduce the risk of surface water intrusions. This is illustrated in a vertical cross section of the upper section of a producing extraction well set into the grade (lateral continuous line) above the static water level (discontinuous line) within a porous formation (PF) which would interact with the well through biofouled voids (see Figure 9.73). The common strategies used to prevent the well from being compromised by over grade flooding are shown in the diagram. These preventions include building up of the well head (WH) to ensure that it is above possible flooding risks (upper dotted line and vertical arrows). Artesian flow is diverted through a side valve (SV) with a backflow valve (BFV) to prevent water entering the well head

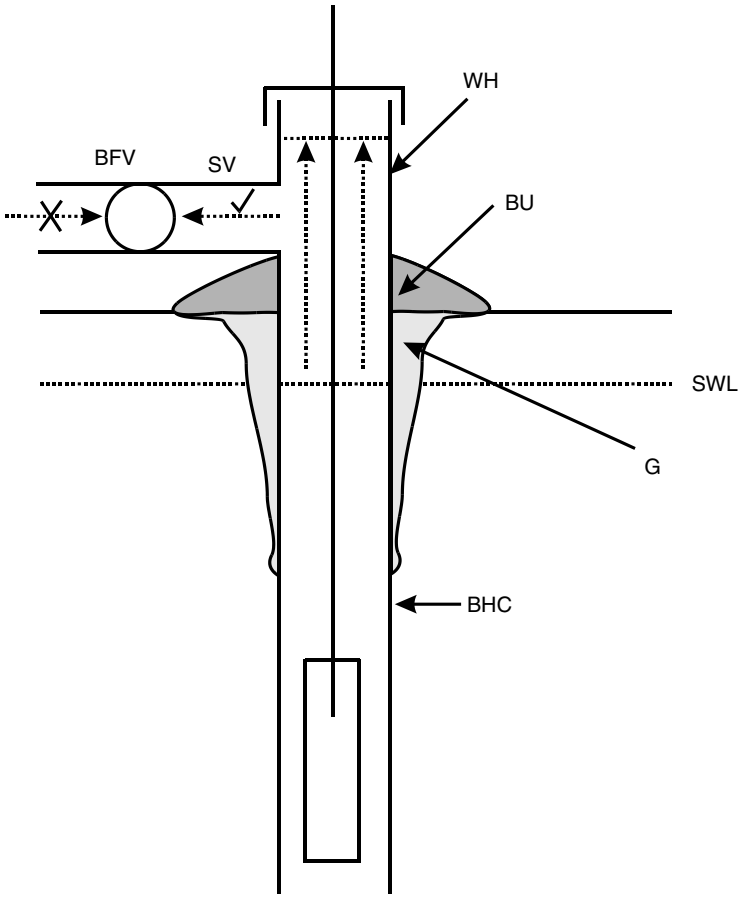

FIGURE 9.74 Schematic of the methods employed to reduce the risks to well susceptible to compromise through by over surface flooding.

during flood conditions. Two methods illustrating the limiting of water egress into the well are the use of grouting (G) below grade and a buffer material above grade (BU).

9.75 IMPACT OF FLOODING ON POSITION OF THE REDOX FRONT AROUND WATER WELLS

In Figure 9.75, the effect of flooding (over grade) and through elevated water table impacts that are showing the influence on the normal position of the redox and biomass fronts (RFBF) and at the peak effect of the flooding (dashed line) with arrows showing the movements that would be occurring in the redox front during the flooding. The upper left diagram shows a well set below grade (G) with a static water level (SWL) with the redox front controlled by the biomass formation. In the upper right diagram, the well has become subjected to deluge by floodwater (FW, wavy line above grade) and oxygen (O_2) now enters with the flood water

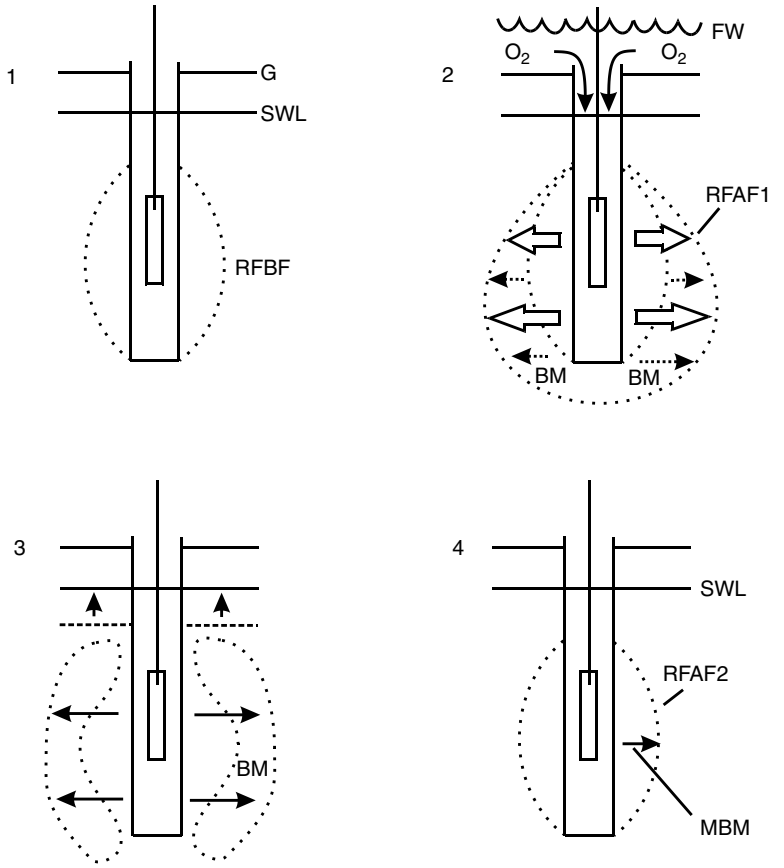

FIGURE 9.75 Impact diagram showing the effect of flooding on the redox fronts associated with vertical extraction wells.

causing the biomass (BM) to retreat away from the borehole (RFAF1). This flooding would cause the static water level to rise towards grade (lower left diagram) and the redox front to reposition further away from the well (RFAF2). This migration of the biomass along with the redox front would be temporary and the well would restabilize once the flooding conditions had passed. For the well there would therefore have been a period of postflood trauma.

9.76 IMPACT OF OIL AND GAS WELLS ON NEIGHBORING WATER WELLS

This is an age (1999–2007 CE) that the spot prices of hydrocarbons (as petroleum products and natural gas) have soared. There are two impacts of these events. First there has been an increasing density of operating oil and gas wells over known reserves. Second water well owners are becoming more sensitive to the potential impacts that these oil and gas wells may be having on their water wells. It is very easy

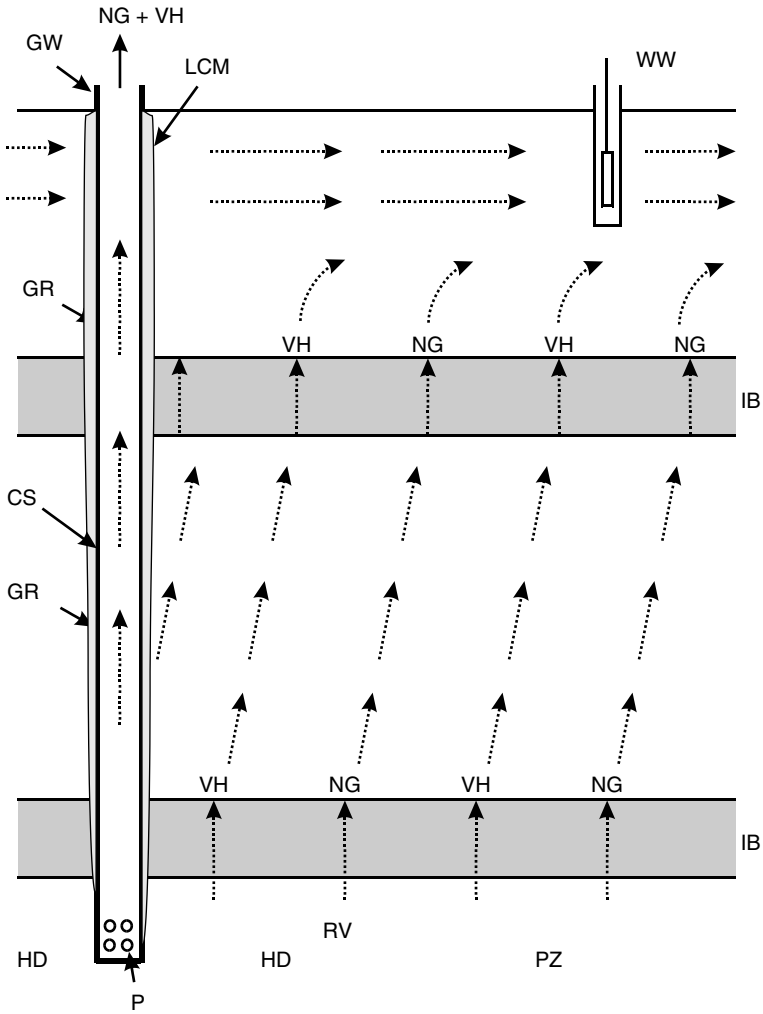

FIGURE 9.76 Schematic of the risks to water wells from neighboring oil and gas wells.

to view at an oil or gas well that has been installed some distance away and blame that oil and gas well for the problems the owner is experiencing in the water well. Figure 9.76 illustrates the installation of WW directly over hydrocarbon deposits (HD) reservoir. This reservoir is releasing natural gas (NG) and volatile hydrocarbons (VH) that are moving upwards and outwards through the groundwater flows (horizontal dotted arrows). Geological impermeable barriers commonly (shaded blocks) intercede with the movements associated with the NG and VH flows but eventually these can arrive at the redox fronts around WW causing challenges. To the left of WW is a gas extraction well (GW) drilled and developed specifically to exploit the HD. During drilling and development, loss control materials (LCM) are normally employed to restrict any movement and natural gas (NG) and volatile

hydrocarbons (VH) during development. Secondarily grouting (GR) is employed to seal the casing (CS) down the producing zone (PZ). Properly installed, the gas well should not contribute to the deterioration of the water well (WW) beyond that which is naturally occurring through the movement of NG and VH from the PZ to the surface environment where these products would be, for the most part, degraded in the biomass growing at the redox front.

It is only when this biomass become saturated with these materials and becomes stressed that these products can emerge in the water well as such. It may therefore be expected that the impacts of gas wells cannot be expected to have either an immediate or a delayed sudden impact on neighboring water wells. Rather the symptoms that this would be happening may be found in the biomass forming the living filters around the water well. Here significant concentrations of VH and/or natural gas are likely to be detectable by chemical analysis and by increased levels of bacterial activity (aerobic heterotrophs detected using the HAB-BART tester).

9.77 THEORETICAL FUNCTIONING OF BART TESTER

Illustration of the principle environments that are generated in a biological activity reaction test BART tester once the 15 mL of water sample (WS) has been added. In Figure 9.77 only the inner vial (IV) and cap (C) are shown for the BART tester with each of the specific environments are defined by zone. These include the following going from the BART ball (BB) descending to the base of the tester: air-surface interface (ASI) with the surfaces created by the ball and water surface (highly oxidative); wall to surface of the ball interface (BB), bottom of the BART ball hemisphere is initially highly oxidative and gas bubbles (GB) can be trapped here temporarily; the oxidation–reduction front (RF) encourages the growth of strictly aerobic bacteria (tends to move up rapidly once activities begin); RF that is commonly the site for the greater amount of microbial activity; reductive side of the redox front where there is less microbial activity and a greater potential for gas production; nutrient diffusion front that is moving up from the basal cone generating a greater level of microbial activity (bioaccumulation and gas generation): in the base cone of the tester are the chemical nutrients (BCN) that diffuse up slowly stimulating microbial activity. As the chemicals diffuse upwards they create two additional zones beneath the redox front. The lower zone is crated as the nutrients diffuse to form a front (NDF) immediately above the cone of the tester. Above this now forms a reductive front (RRF) which would support the growth of some anaerobic bacteria. Often gas production (carbon dioxide and methane) can become centered in this region with gases being released as bubbles often with slime strings flowing after the bubble (very common in the SRB-BART tester).

9.78 POSITIONING OF BIOMASS AROUND SLOTTED SCREENS IN BIOFOULING WATER WELLS

Video camera logging gives an understanding of the growths occurring within the water column of a well and outwards into the slots but not beyond, Figure 9.78 shows the manner in which biofouling can occur around six slotted screens. This is

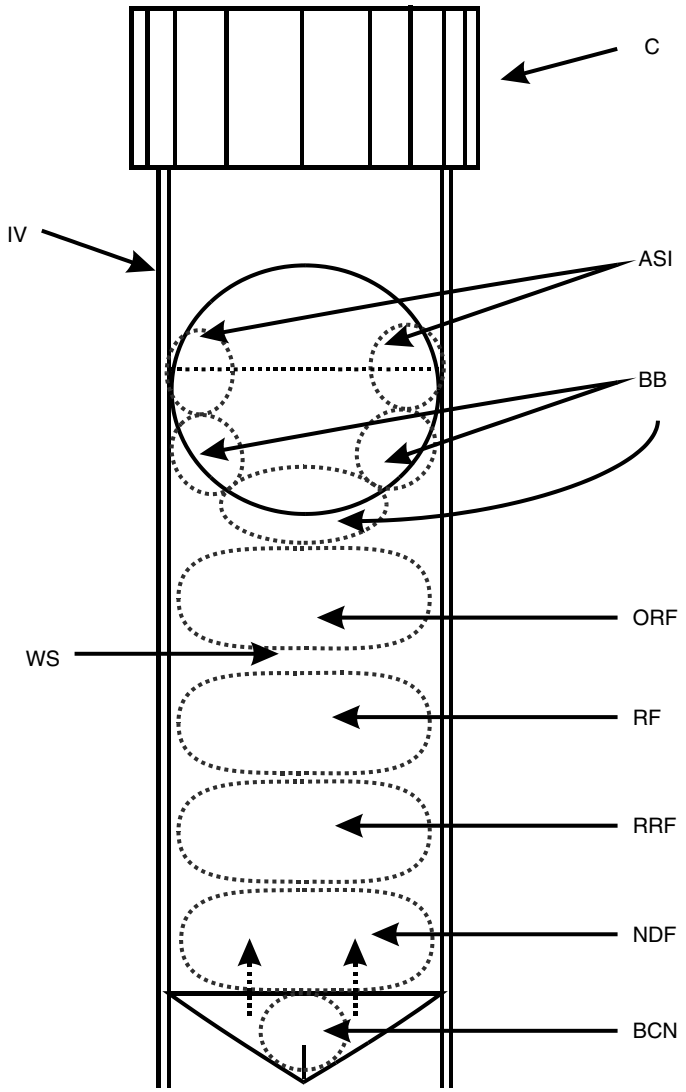

FIGURE 9.77 Theoretical location of environments (as distinct zones) generated in the BART tester once the sample has been dispensed.

shown through six cross sections of a vertical section of an extraction well exhibiting the six stages in the plugging process:

1. Illustrates the well (W) with static water level (SWL) and slotted screen (SS) but with no biomass present.
2. Shows the well during development when a large biomass growth (BG) occurs close to the screen causing a colloidal type of blocking that dissipates by the end of development.

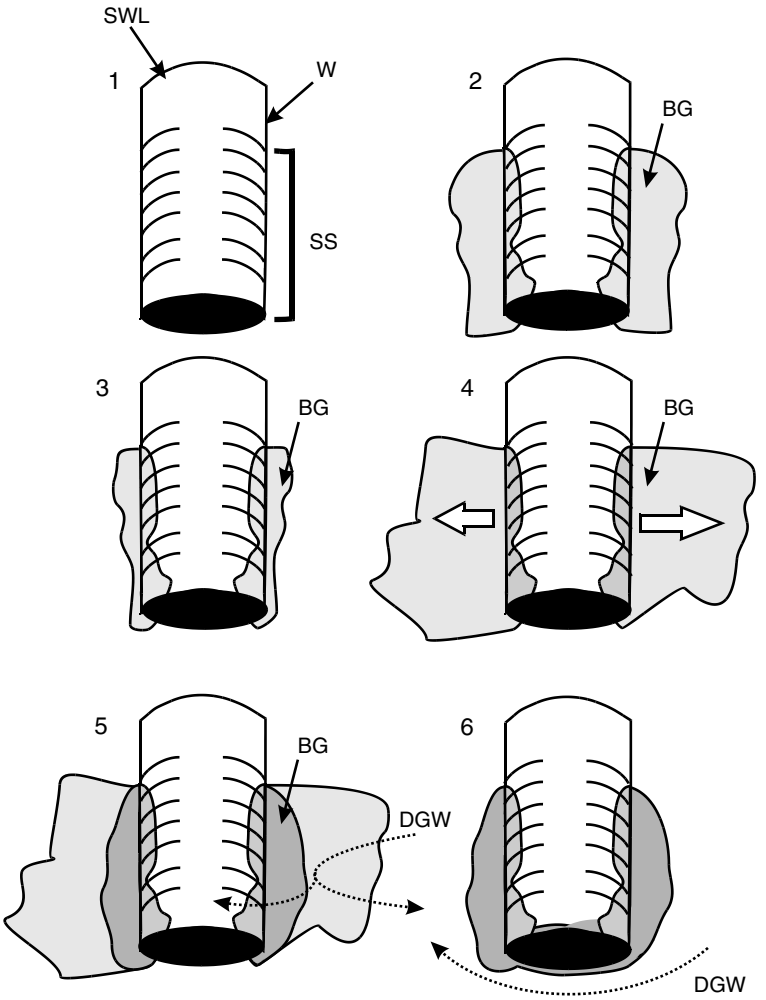

FIGURE 9.78 Diagrammatic presentation of the shifts in biomass location and form during the plugging of water wells.

3. Follows development and the BG are diffuse and located close to the borehole.
4. Biomass now has grown sufficiently (white arrows) to plug (>60%) regions in the porous media causing diversion in water flow.
5. Biomass expands and becomes denser due to the bioaccumulations of metals and/or carbonates, directing groundwater flow (DGW) now significantly diverted (dotted arrows).
6. There is now such an intense biomass that is thick enough to plug off all entrances through the slots. All of the groundwater flow is now blocked from entering the slots and the water flows are diverted.

9.79 TREATMENT SEQUENCES IN BIOFOULED WATER WELL REGENERATION

There are many challenges in successfully treating plugging water wells that has a growing biomass forming around the well. Treatments should be designed to effectively remove the bulk of the biomass and return performance closer to the original developed form. The prime objective is to regenerate the well so that it can then become managed in a sustainable manner.

Figure 9.79 forms the basis for illustrating the impact of regeneration, the stages of treatment commonly involved are:

SK to cause physical and chemical trauma in the bacterial communities formed within the growing biomass.

Disrupt where the biomass is broken up by a suitable mixture of physical and chemical agents.

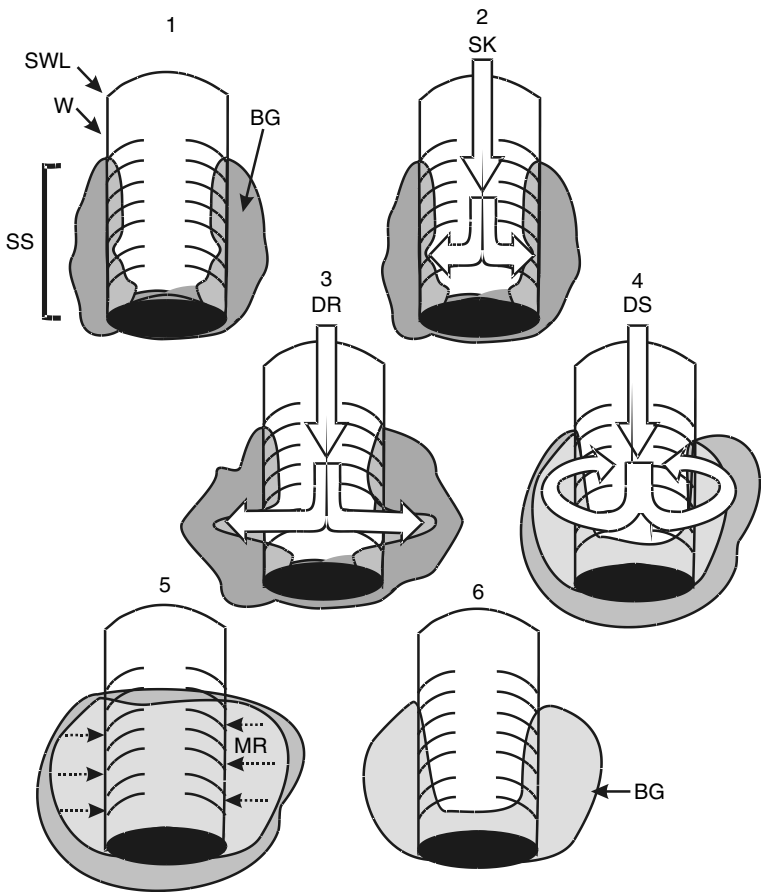

FIGURE 9.79 Impact showing the manner in which the indigenous biomass are impacted by successful regenerations.

Disperse (DS) which is more commonly a physical event involving the forced removal of the particulates generated by the DR of the biomass.

The well (W) is shown to have a static water level (SWL) a slotted screen (SS) along with the generating biomass growths (BG). These three treatments are shown to sequentially have a graduated impact on the biomass (1). Shock (SK), as (2) has a limited impact on the biomass closer to the borehole causing these microbes to go into trauma or be killed. (3) disruption (DR) now extends the treatment deeper into the biomass causing the biomass to collapse. (4) dispersion (DS) illustrates the use of physical action and additional chemistry and heat to cause the total collapse and removal of the shattered biomass. Next comes (5) which shows the well immediately after regeneration with the surviving biomass beginning to move in and reform due to the entry of microbes relocating (MR) from beyond the sphere of treatment impact (arrows) and also through the growth of microbes that had survived the treatment (double circles). Note that the effects of SK, DR, and DS, when successful, will leave the well with many cleaned surfaces to which microbes can attach and grow, and also food in the form of dead biomass would also be available to cause the first surge in posttreatment microbial activity. Finally, the well after completion (6) of the DS should now have a smaller amount of biomass present commonly further out from the well.

A Microbiological Test Methods

A.1 INTRODUCTION

Testing for microorganisms in water can involve a range of possible approaches from a direct microscopic examination of the water sample to look for cells, through to cultural methods in which some (but rarely all) of the microbes can be counted if they respond to the testing method, as well as the determination of the levels of specific chemicals in the cell or the sample. Currently these latter types of tests are dominated by the determination of the levels of high energy stored within the cells as adenosine triphosphate (ATP); and the genetic identification of specific genomes associated with particular species or communities of bacteria. Test methods have arisen out of history as different approaches have been used to detect and enumerate (count) the various species of bacteria thought to be significant.

A.2 HISTORICAL BACKGROUND

Microbiology as a discipline grew in a ragged manner from the seventeenth century to the latter part of the nineteenth century, impacted by squabbles over jurisdiction between zoologists and botanists, a general denial of the existence of microorganisms by chemists and an absolute ban by most religions on any consideration of life as such even existing beyond the range of human vision. Furthermore it was thought during the nineteenth century that if such life forces did exist then they were the work of the devil. Chemists thrived at this time on the concepts that all diseases were miasmic and were chemical in origin.

Microbiology was nurtured in the latter part of the nineteenth century by a series of signal events that could not be denied by chemists, physicists, theologians, and society in general. A number of diseases were clearly shown to be microbial in origin with anthrax being demonstrated as being a bacterially induced disease (thank you Koch) while rabies was shown to be a viral disease (thank you Pasteur). This led to the golden age of microbiology where new species were discovered on almost a daily basis and the industry flourished, particularly in the health and food sectors. Many of the basic cultural and physiological practices were laid down at that time and remain in use to this day. Perhaps of all of the techniques developed, it was the use of agar media and the Petri dish that had the greatest impacts. These techniques allowed the culture of isolated colonies of microorganisms in a manner that gave

confidence to the outcome of testing. The shortcoming of agar is that it has a very selective nature that was generally ignored in favor of the advantages of identifying the colonies that did grow.

By the start of World War I, there was now a large knowledge base on significant microorganisms (generally those known to cause disease). Two conflicts emerged. One conflict was generated by chemists who believed that the control of these microbial pathogens could be achieved completely through the use of chemicals. This had been validated by Lister with the introduction of antiseptic surgery using halogens or phenols. Improved understanding then led to the rise of aseptic surgery which did not involve chemicals. Erhrlich promoted the idea that all pathogens could be controlled chemically using synthetically derived organic compounds. This was confirmed in the 1930s by the sulphonamide drugs. The other conflict was generated by the many reports of microorganisms producing antibiotics but none were seriously developed in the early twentieth century. Even Fleming, with the discovery in the late 1920s of penicillin, initially failed to raise the awareness of the potency for applying antibiotics to control infections. World War II changed these attitudes to now accept the suitability of microbially derived antibiotics. This developed into a major industry.

From the 1980s to the present time, the focus has changed back to predominantly chemical approaches with the romance of discovering the significance of the structure of DNA although its true functioning remains elusive. Today microbiology has been distorted to favor molecular evaluations rather than to determine consortial, community, or even single species. Today microbiology uses a combination of traditional laboratory-based detection and enumeration procedures based on the cultivation of the microbes in the sample, and modern sophisticated chemically based methods that in general require a significant cost and advanced training to allow the testing to be achieved with precision.

Thus there is a new confusing array of microbiological tests that can be performed on a water sample to determine the microbial content. They can be categorized into three main approaches:

1. Direct examination of the sample using some type of light from regular light to pulsing red laser beams.
2. Cultivation methods that selectively grow specific types of microbes and records their presence as predicted population or activity levels.
3. Biochemical evaluation of specific chemicals such as genes, enzymes, metabolites, or stored materials.

These three approaches are summarized below.

A.3 DIRECT EXAMINATION OF WATER SAMPLES FOR MICROORGANISMS

Direct examinations of water samples for microbial presence have a challenge in that even if the water had a high clarity it would remain very difficult to see any

microorganisms using a light powered microscope. Here it would be necessary to stain the cells or the background in order to see the cell (when stained) or the outline of the cells (when the background is stained using negative staining). With these techniques we have the disadvantage of the human eye which tends to gravitate to unusual shapes and forms.

Three simple cultural techniques that can be used to determine the presence of active bacteria by direct examination are the dip paddle technique, the slope BART tester in which the agar surface is exposed to the water sample prior to incubation and the regular BART™ tester where the 15 mL water sample is added to the tester and incubated to determine the activity level in the sample. Here the activity of the microorganisms within the sample can be assessed visually after incubation. More direct visual examination involves the microscope. This may give a distorted picture since the samples with a dominance of *Gallionella* (which has a large imposing ribbon-like tail) is easily seen and recognized while microorganisms skulking in the colloids are often ignored even though they might have a greater number and mass of living cells.

In modern microbiology the pulsing laser beam offers some significant advantages in that it will actually measure the suspended biocolloidal material within the sample. Standard techniques commonly use a laser particle counter (LPC) that is able to not only measure the size of individual colloidal particles but also compute the total suspended solids (TSS). In the laboratory the application of the LPC technology makes a very good start to the examination of a water sample for microorganisms. If the TSS is less than 0.02 ppm and virtually no particle is found larger than 2 μm then the probability of significant microbial populations in that sample is remote and further testing may be simply unnecessary. One risk with the LPC technology is that the biocolloids may behave like little submarines and float at specific depths in the sample. If the pulsing laser beam fails to go through the water sample where the biocolloids are then a false negative would be recorded. Once the TSS goes above 0.08 ppm and on up to 1.5 ppm then significant biocolloids are present and normally the size of the particles get larger reaching, on average, as much as 4–12 μm. This is the range that would commonly be recorded when an LPC is performed on a water sample from a biofouling water well.

A.4 CULTIVATION METHODS APPLICABLE TO DETECTION OF MICROORGANISMS IN WATER

From the middle of the nineteenth century, culture techniques were applied to detect microorganisms in water. Standard methods of that time included such things as counting what colonies would grow on a slice of potato, culturing in a clear meat broth, and streaking it out onto a strip of gelatin. While these methods were crude, they did give a differentiation between samples that had little to no microbial activity from those samples that supported significant microbial populations. Methods of the simple "kitchen sink" variety have been developed by people who wanted an answer to the question "Are there significant numbers of iron bacteria in my water sample?"

When working in the field remote from laboratory support facilities, a range of simple techniques have been developed which can give an indication of whether there are microbially driven events occurring. These techniques are by nature very simple and in some cases involve locally available material. Incubation is at room temperature as a matter of convenience. There are four tests commonly used for the low-cost first-reaction testing developed by Rodina, Cholodny, Grainge and Lund, and George Alford. All employ simple techniques that can found around the home, at a restaurant, or even in the bar!

Rodina Test. This test was described in 1965 and it functions through the ability of some IRB to generate a flocculant type of growth when the water is subjected to very aerobic conditions. The test itself consists of pouring water into a wide-necked flask or bottle and leaving the water to sit open overnight without being capped in any way. Do not fill the container to a depth of more than 10 cm (4 in.). Here, the large surface area generated in the bottle gives plenty of area through which oxygen can diffuse into the water. This increasing oxygen concentration to saturation causes some IRB to form into flakes that resemble discolored cotton wool in appearance. Where this occurs, the presence of IRB can be suspected and confirmed by a direct microscopic examination of these wool-like "growths."

Cholodny Test. In 1953, this method was described in which the IRB could be seen when these organisms attached to glass. The method calls for some of the water and any sediment recovered with the water sample to be placed in a jar. A cork is then floated on the surface of the water sample with one or more pieces of glass (such as bacteriological slides or small pieces of rectangular glass) attached to the underside. As the water clears through sedimentation, the glass attached to the floating cork becomes visible. The development of any "rust spots" or "cotton-like accumulates" either on the surface of the sediment or on the glass inserts can be taken to indicate the presence of IRB. Where there were very large and aggressive populations of IRB, such events could occur in less than 24 h of incubation. Where identification of the IRB was desirable, the glass inserts could be removed from the cork, detached, and air-dried for microscopic examination.

Grainge and Lund. This test identified with the need to develop a very simple test in which iron is added to the water sample in such a way that the activity of IRB could be easily identified. This technique was described in 1969 and has been recommended as a monitoring procedure for the effectiveness of control programs. A clean soft steel washer (preferably chemically cleaned) is placed in a conical flask (e.g., Erlenmeyer flask) along with a white extruded plastic rod (e.g., stir stick) which is positioned so that it is roughly vertical. The water sample is now added to sufficiently cover the washer and leave the end of the plastic rod sticking out of the water. After being left for 2 days, the water line around the plastic rod is examined for any translucent string-like (filamentous) growths. Over time this growth may develop a brown tinge due to iron accumulation. This is taken to be a positive indication of the presence of IRB.

George Alford. This is a quick and crude test for IRB which involves the use of materials that are easily at hand to determine whether IRB are present or not. This method, developed in 1980, involves the admission of a non-galvanized iron washer

to the water sample along with a carbon source (ethanol) to stimulate the activity of IRB. To test a water sample, 150 mL of the sample is added to a clean glass and the washer dropped into the water now comes to rest at the bottom. Two drops of Jack Daniels whiskey are now added to the water ("one drop for me and one drop for the bugs," George Alford, personal communication). Once the Jack Daniels has been added to the sample the glass is loosely capped with an aluminum foil (to reduce evaporation). Incubation is better in a warm place such as on top of a refrigerator or on a shelf or windowsill. A positive reaction is recognized by either a "fuzzy" growth around the washer or metallic "floaters" in the water. In both the cases, IRB are actively either attaching directly to the source of iron (i.e., the washer) or accumulating the dissolved iron within suspended particulate masses (i.e., the floaters). This can happen overnight in a war, environment.

These methods are commonly supplemented by observing what is actually happening at the well head with brown or black slimes, grittiness in the sediment, and the smells all playing a role in trying to determine the origin for the problems with the water well. Once the water sample gets to the laboratory then it becomes possible to stain the sample in order to see any microbes that might be lurking there or whether iron has been accumulated into the slime-like or gritty sediment. There are a number of methods that have been in use to determine this. They include the methods of Olanczuk-Neyman, Meyers, Leuschow, and Mackenthum, and the negative wet mount. These are described below.

Olanczuk-Neyman Method. This methodology was developed in Poland while working on IRB fouling of water intakes. Here, a staining technique was employed which used membrane filtration to concentrate the suspended particulates prior to the staining with a modification of a procedure developed by Rodina. Under low power magnification, the bacteria cells will appear to be stained red while the iron deposits will be stained blue. Under oil immersion microscopic examination, the membrane filter will become transparent when saturated with the oil. This makes the observation of the IRB much easier. The Olanczuk-Neyman method calls for the numbers of IRB to be enumerated using 100 microscopic fields selected randomly and this is related back to the number of IRB/mL. It should be remembered that much of the material being viewed microscopically is dead including the stalks of *Gallionella*.

Meyers Stain. Meyers described a staining technique for IRB in 1958 which has been found to produce superior results to the other techniques presently available. Like the Olanczuk-Neyman method, this technique stains the bacterial cells red while the iron deposits are blue with better differentiation. This technique is primarily recommended for use with water samples which are showing some signs of a possible infestation (e.g., the water is discolored to a yellow, orange, or brown hue, the water has a distinct colored deposit which may have an indistinct or fuzzy outline). This technique is not quantitative but does allow a variety of the IRB to be identified as present (or absent) and a relative relationship to be expressed between the species observed. The limitation of the technique is that it does not necessarily allow a clear observation of the bacteria which may have accumulated iron within irregular amorphous structures. Additionally, the technique is not suitable for waters

with a low IRB population. Such waters are best stained using the Olanczuk-Neyman or the Leuschow and Mackenthum membrane filter methods.

Leuschow and Mackenthum Direct Membrane Filtration (*MF*) *Technique*. This technique was described in 1962 for the investigation of waters which had a low number of IRB. It does not involve staining but rather a direct observation of the bacteria that have been entrapped on the membrane filter which has been dried and rendered transparent with immersion oil. It is restricted, as a technique, to only those bacteria which are occurring in large and/or distinctive structures and are pigmented (commonly by the entrapped ferric-iron oxides). Various common forms of stalked and sheathed IRB may be easily seen by this technique even when they are present in the water in relatively low numbers. In Wisconsin, Leuschow, and Mackenthum recovered IRB from 55% of the well waters tested with *Gallionella* and/or *Leptothrix* being the most common dominant IRB identified by this method. In the reddish turbid waters, counts by this method reached $> 10^7$ IRB cells/mL.

Negative Wet Mount Stain. One major problem with the staining techniques is that the "edge" of bacterial cells, particles, sheaths, and stalks may be diffuse and difficult to observe. Additionally, where the bacteria are not pigmented or have not accumulated iron, the cells may be transparent to light and not be identifiable. The negative stain uses a different concept in that the background is stained black while the bacterial cells remain unstained. Thus, the cells now stand out as being illuminated zones within a darkened (stained) background. This is achieved using nigrosin (commonly called Indian ink) which is an acidic stain. Such stains do not penetrate and stain bacterial cells due to the fact that both the stain and the bacterial cells are both negatively charged. The stains do tend to produce a deposit around the cells which forms into a dark background in which the bacteria appear as clear, unstained regions which relate to the shape of the cells. Where nigrosin is not available, Indian ink (25% aqueous solution) may be used. This negative stain is more satisfactory for waters which are showing some visible signals of possible microbial presences (discoloration and clouding) and has the advantage that all types of bacteria may be observable by this technique. There are a number of mechanisms to obtain the sample for the negative stain. Approximately 0.2 mL of suspension is used in this staining technique. Turbid water or a centrifuged pellet would probably have too many cells to be clearly definable and recognizable. On the other hand, a regular "clear" water sample may have too few cells to be conveniently observed. In either event, some dilution (into a sterile Ringers solution) or concentration (by passive settlement or centrifugation) may be needed to conveniently view a dispersed microbial mass. Trial and error may be required to find the appropriate dilution or concentration of the water sample.

In this method, cells will appear to be either transparent (if there has been no excessive accumulation of iron) or orange to brown where iron has been accumulated. The background will appear to be pale blue. Where there is a "tight" form of extracellular polymeric substances (EPS) around the cell (e.g., in the form of a capsule, sheath or a thick coat of EPS), this will form the distinct boundary rather than the cell itself. With careful focusing and adjustment of the light, the cells can sometimes be seen within the extracellular structures. Dry mount negative stains of

samples can be made into archival copies which can then be kept and viewed at leisure (the wet mount preparations will dry out rendering them of little value). Where this is desirable, the slide should be left to air dry.

A.5 USE OF AGAR PLATES TO DETERMINE BACTERIAL ACTIVITIES

One of the major problems facing microbiological laboratories undertaking the standard bacterial counts is that the laboratories are often faced with the serious problem of large discrepancies between microscopic and plate counts, especially for cells isolated from natural environments. This means that the precision does not exist in the manner that can be comfortably expected for most chemical analytical procedures. Errors can sometimes extend in bacterial counting through orders of magnitude of difference that cannot be accepted in the modern environmentally regulated society. Today the agar plate technique remains a recognized standard but this is gradually being challenged by various, more precise cultivation techniques and the application of refined biochemical techniques.

The most common technique used and generally recognized as being valid is the extinction dilution/spreadplate enumeration (as colony forming units, cfu/mL, or per 100 mL) using a variety of agar media. There has been, over the past 50 years, a growing debate as to which of the vast array of culture media are the most suitable. While debate can be healthy, it is centered on the use of an agar gel technique that has a number of significant limitations

1. The quality of the agar, commonly as a natural sea weed product, can be somewhat variable and affect the nature of the microbes able to grow on the medium;
2. The water inside the agar, that has to be extracted by the microbes in order for them to become more active and grow, is tightly bound and it requires energy expenditures to be extracted;
3. The environment generated by the agar becomes strongly oxidative which can be a deterrent to the growth of some oxygen sensitive microbes;
4. Agar has to be heated in order to become molten and some techniques (e.g., poured plate) expose the microbes in the sample to 45°C or higher as the agar sets.

In spite of the shortcomings of the agar techniques, this debate has centered on the most appropriate culture medium for the enumeration of aerobic heterotrophs, hygiene risk indicator bacteria (e.g., coliforms, enterococci, *Clostridium welchii* and species of *Klebsiella, Acinetobacter*, and *Aeromonas*), iron related bacteria, biodegraders, sulfate-reducing bacteria, and anaerobic bacteria. Considerations also range through the use of the MF technique (where there are low populations in the cfu/100 mL range) and the correct methodology for extinction dilution needed when there are high populations in the cfu/mL range.

Methodologies tend to ignore these serious concerns expressed above which causes serious discrepancies to be observed between microscopic (higher numbers) and spreadplate counts (lower numbers). These concerns would relate to the potential loss of precision in the estimation of microbial cell populations in a water sample using the agar spreadplate techniques. However, today there remains a heavy reliance on the relative value of the population numbers recorded in a sequence of samples and the cfu/mL generated that has been considered by many users to closely reflect the number of cells at least in the comparative sense.

In conducting a spreadplate enumeration from a water sample, there are a number of stages that are involved after the selection of the appropriate agar culture medium:

1. Dispersing the particles within the sample by some form of agitation (e.g., vortexing).
2. Serial dilution of the water.
3. Dispensing of diluents onto the agar surfaces.
4. Drying of the agar surface (optional).
5. Incubation under the correct temperature and atmosphere (reductive or oxidative) to allow any growth to occur.
6. Counting the grown colonies which are visible.
7. Computing the cfu/mL or cfu/100 mL.

A.6 SELECTION OF AGAR MEDIA

Agar itself is a galactoside obtained from certain marine red algae (seaweeds). Most microorganisms are incapable of degrading agar–agar. It is used at concentrations of between 1.0 and 1.8% (w/v) depending upon the formulation of the medium and the quality of the agar–agar used. Lower quality agar–agar contains up to 0.5% phosphorus (as P) while higher qualities contain less than 0.2% P. This translates into a potential supplementation of the agar medium with up to 75 parts per million (ppm) of phosphorus, respectively. This would form a significant additional (and uncontrolled) nutritional base for microbial growth and activities. Indeed, one such medium (water agar) utilizes just the nutrients inherently present in the agar–agar and the water used to make the medium. On occasions where the microflora has a significant (if not dominant) organo-sensitive component, the water agar may give the highest counts of cfu/mL when compared with other recommended media.

Agar–agar was originally selected because of two very favorable factors. First, the agar–agar was not degraded by most types of microorganisms and therefore relatively stable (when compared to gelatin). Secondly, the agar–agar would remain as a "solid" gel at temperatures up to boiling point (i.e., $>90°C$) which would allow incubation in the thermophilic range; and yet would not set from the molten state until 46°C. This latter feature allows microbial suspensions to be mixed into the setting agar–agar so that a greater dispersion occurs in the colonies that are formed

subsequently. This is known as the pour plate technique. This method is now not widely used for environmental samples because the heat shock caused by adding the organisms to the cooling molten agar–agar at $>45°C$ can be traumatic.

In the cultural techniques using agar–agar, the water and supplemented chemicals are dispersed into the agar–agar which is then allowed to set. For a colony to form, the organisms have to "mine" both the water and these chemicals from the agar–agar base. If organisms are not able to extract adequate water and nutrients from the gel base, colonial growth would not occur. This may be a major factor restricting the range of microorganisms able to grow on agar culture media.

In higher relative humidities where the agar medium is not subject to radical evaporation rates and drying out, 15 mL is a common volume to dispense molten agar media into standard Petri dishes. However, in dry climates such as is commonly experienced in the North American prairies greater volumes of agar (20–25 mL) may be dispensed to compensate for the losses through evaporation which also causes the chemicals within the agar medium to become concentrated and possibly inhibitory. "Bagging" the poured plates in a plastic bag may not function well since water may condense on the walls of the plastic bag and then drip back into the dish causing contamination and erroneous results. Inoculated agar plates are incubated upside down (agar facing downwards) so that water cannot condense onto the upper inner surfaces of the lid and drip back down onto the growing colonies.

Each agar culture medium is developed to enumerate a particular spectrum of the microflora in the water and no single medium is capable of stimulating the active growth of all of the microorganisms that could conceivably be present. In other words, all agar tests are, by their very nature, selective. There are however, some broad spectrum and selective forms of agar media which serve the very different purposes required for detection and enumeration of different microbes. The broad spectrum media generate a set of nutritional conditions in which a wide variety of microorganisms may be able to flourish (e.g., the heterotrophic bacteria). Selective culture media implement the use of inhibitory chemicals (e.g., antibiotics), marker dyes and restrictive nutritional regimes which encourage and support the growth of a narrow spectrum of microorganisms. Various agar media are listed below and frequently are recognized by their acronym rather than by their original descriptive label.

AA agar. A very selective medium for the Gram negative strictly aerobic heterotrophic bacteria and, in particular, the genus *Pseudomonas*. Colonies are commonly discrete and may be pigmented or if the pigments are water soluble, these may diffuse into the underlying agar medium.

Brain heart infusion agar/4 (*BHI/4*). This quarter strength brain heart infusion agar has been found, in practice, to support excellent colonial growths of a broader spectrum of bacteria than the R2A agar medium. Colonies tend to grow more rapidly and to a larger size. It is a very useful medium to obtain a generalized enumeration of aerobic bacteria. If placed under anaerobic conditions (e.g., through the use of an anaerobic jar and gas pack), colony counts for anaerobic bacteria can be obtained.

Czapek-Dox agar (*CD*). This is a broad spectrum medium for culturing many of the fungi (molds). This medium is particularly suitable for the culture and enumeration of *Aspergillus*, *Penicillium*, and other related fungi. Heavy fungal spore

populations may be present in groundwaters which have interfaced with unsaturated zones (e.g., via recharge) with some level of organic input (e.g., from sewage lagoons or oxidation ponds). Typical fungal colonies appear to have a rough almost cotton-like appearance which spreads out over the surface of the agar. Visibly distinctive bodies within the mass of growth (called a mycelium) may form the sporing bodies that sometimes look like pimples (spores) in the "wool" (mycelium).

LES Endo agar. This is a traditional medium that was developed for the enumeration of total coliforms. It is a complex medium containing sodium desoxycholate (a bile salt) which will inhibit many of the bacteria which are not commonly found in the intestinal tract. Lactose is the major carbohydrate (sugar) which is fermented by a relatively narrow spectrum of bacteria which includes the coliforms. Typical coliforms have a colony with a golden-green metallic sheen which develops within 24 h. This sheen may be centrally located, around the periphery or cover the whole colony. Often used as a basal agar for the incubation of membrane filters. It is generally considered that a valid colony count will be one where there are between 20 and 80 typical colonies per filter and no aberrant atypical overgrowths.

Lipovitellin-salt-mannitol agar. This is a medium used to determine the presence of *Staphylococcus aureus*. This bacterium is commonly a concern in confined recreational waters where bathing is commonly practiced. When present, the colonies formed are surrounded by a yellow zone with opacity. The opaqueness relates to activity which correlates to the presence of coagulase-positive staphylococci that are considered to be more of a health risk.

M-FCIC agar. This is a medium which is specific for the genus *Klebsiella*. Typical colonies of *Klebsiella* produce a blue to bluish-grey colony. This medium is used to determine the presence of this genus in waters by membrane filtration. Atypical colonies are beige to brown in color. *Serratia marcescens* will also grow but will be distinctive by producing a pink colony.

M-HPC agar. This is an agar medium which has been developed for use with the membrane filtration or spreadplate evaluation for heterotrophic bacteria. It is an unusual medium in that it contains glycerol and gelatin as two major sources of nutrients.

Mn agar. This medium incorporates both reduced ferrous and manganous forms, along with citrate to encourage the growth of iron related bacteria. Colonies typical of IRB generally are brown to dark brown or even black and may be mucoid (sticky looking), granular forms that may be irregular or whorled over the agar surface.

M-PA agar. This is a medium used in conjunction with membrane filtration to detect the presence and numbers of *Pseudomonas aeruginosa*. Typical colonies are flat in profile with brownish to greenish-black centers which fades out towards the edge of the colony.

Pfizer selective enterococcus agar. This is a medium which allows the differential culture of the enterococci (a.k.a. *Streptococcus faecalis* group). These bacteria form brownish-black colonies with brown halos.

Plate count agar (*peptone glucose yeast agar*). This has been a major medium used to enumerate heterotrophic bacteria by the spreadplate method.

Potato dextrose agar (*PDA*). This medium allows a broad spectrum enumeration of both yeast and fungi (molds). Yeast tends to form large (3–6 mm diameter) colonies which have a domed profile and may be pigmented a pink color. Some yeast also emit fruity odors (esters) which can be very distinctive. A broad range of fungi will also grow well on this medium often giving cotton wool-like appearances that can resemble impressed felt.

R2A agar. This is a more complex medium which has been found to support a broad range of aquatic heterotrophic bacteria. It is somewhat more difficult to prepare. There are a number of varieties of this medium that laboratories use. The R2A medium is being used more widely with spreadplate techniques to determine the total bacterial count and is particularly supportive of many of the pseudomonad bacteria which frequently cause problems in controlled recreational water systems (e.g., whirlpools and swimming pools). Some laboratories use dilutions of this medium and claim to obtain greater recoveries (i.e., higher colony counts). Common dilutions are R2A/10 and R2A/100. R2A will also support the growth of some *Actinomycetes* and, in particular, the *Streptomyces* (a common source of the earthy musty odors called geosmins that are associated sometimes with taste and odor problems in water).

Starch-casein agar. This is an agar medium developed for the selective enumeration of mycelial bacteria (*Actinomycetes*). These bacteria resemble molds producing cotton-like or chalky growths like fungi except that here the growths are tightly bonded to the agar and the texture is often leathery. The fluffy appearance is due to the frequent production of raised spore bodies.

Triple sugar iron agar. This medium was developed for the examination of food products for enteric bacteria but is applicable to groundwaters. Some slime forming bacteria will produce such copious mucoid formations that the slime can fill the void space between the agar and the lid of the Petri dish. It is a useful medium to determine whether enteric bacteria (agar turns yellow when lactose fermentation generates acids; turns red if proteolysis occurs; and black if hydrogen sulfide is produced) or slime forming bacteria (excessive mucoid colonies formed) are present and dominant.

Tryptone glucose extract agar. This is a general medium used for the culture of heterotrophic bacteria. A wide spectrum of heterotrophic bacteria will grow on this medium which is a minor variation of the standard plate count medium. Here, the yeast extract has been replaced by a slightly higher concentration of beef extract.

Winogradski–Regina (*WR*) *agar*. This medium is based on the formulation for culturing iron related bacteria. This medium generates brown colonies for the typical heterotrophic iron related bacteria due to the uptake of iron from the agar medium. Under circumstances where the groundwater is from regions with a high level of organic pollution (e.g., gasoline or solvent plume), biodegraders may also grow on the WR agar medium but will normally produce atypical colony types (commonly white or beige).

A.7 SPREADPLATE ENUMERATION FOR MICROBIAL CONTENT OF WATER

For the last century, the use of agar spreadplates in the enumeration of microbial populations and types have played a major role in the examination of waters and waste waters. In general less regulatory attention has been paid to the microbial content of groundwaters other than the coliform group of bacteria. One major problem with the application of the spreadplate agar enumeration systems is that they tend to be very selective favoring those bacteria that are able to "mine" water from the agar, survive in a very oxidative environment, be resistant to the low relative humidities on the surfaces where the colonies are growing, and be able to grow a large enough colony of cells to be visible and countable (the lower limit for this is 0.1–1.0 mm).

Experience has shown that the agar spreadplate techniques support the growth of less than 5% of the active bacterial species in a water sample because of the restrictions imposed by the technique. In the medical and food industries, the application of the spreadplate (and poured) agar techniques are well established as generating reasonable precision and sensitivity. For the water well industry, there are challenges which reduce the value of the data generated by these techniques. Currently there are two approaches which challenge the value of using agar-based technologies. These are centered, on the one hand, around improved biochemical detection looking for functional (e.g., ATP) or genetic markers, and, on the other hand, through the improvement in the culture of microbes while presenting a more comprehensive range of selective environmental niches (places) where different microbes in the sample can.

A.8 BIOCHEMICALLY BASED DETERMINATION OF MICROORGANISMS IN WATER

Biochemical test methods are generally viewed to be much more specific chemically and generally more accurate for the detection of a specific chemical activity. In general, chemically based tests are viewed to have a greater precision than biologically based cultural methods. There is a large range of biochemical test that have been developed and are used for the identification of specific species of microorganisms. In the application of microbiological test methods, interest is more centered on the activity levels and size of the microbial communities and so this section will address only those techniques that have an application in the water well industry. Within this industry the prime focus is on how large the microbial populations are in, and around, the water well. Additionally, what are the dominant types of microbial communities that are detected as being active?

One "gold standard" that is becoming adopted by many industries is the measurement of ATP. This molecule is the prime site for high energy storage within the cell (that is true of all cells not just microbial!).

ATP, is a chemical that is present in all living cells and forms the major method for energy storage (rather like a car battery) in a cell. ATP assays are used as indirect

measurements of the population of all of the active viable cells in a sample that are using and storing energy. The assay uses the reaction involving ATP and luciferase (an enzyme isolated from the firefly, *Photinus pyralis*) to release light in a quantity that is proportional to the amount of ATP in the sample extracted from the viable cells. The amount of light produced is measured by the number of photons detected using a high sensitivity luminometer which is used to estimate the active bacterial population in a sample. As the amount of ATP varies depending on the type of cells, this has to be taken into account in correlating between the number of photons detected and the number of bacteria present in the sample.

The ATP assay can be performed using instruments such as the MGM Opticomp I Luminometer. Samples for testing have to be sterilized and the ATP released using a special releasing agent. Once the ATP has been released it is placed in a luminometer. At this time the enzyme, luciferase, is injected into the test tube. Over the next ten seconds after injection, the light output (triggered by a reaction between luciferase and ATP) can be measured. The raw data output from the luminometer is given in relative light units. For most applications this data is converted to ATP using a calibration curve specific to the instrument and procedure that relates to the sample origin.

At its most sophisticated level, the ATP assay can give an accurate assessment of the number of functionally active cells in the sample. The type of releasing agent used can allow a differentiation between microbial cells and the cells from protozoa and larger organisms. In its simplest form, it is possible to detect microbial fouling on surfaces and simple swab techniques are widely used in the food industry for this purpose.

BIOLOG® system is a method that employs 96 biochemical tests in separate wells in an incubation tray. Here a reader scans the incubated tray and detects patterns of reactions. These reactions are based upon the manner which the organism under testing reacts with six to eight different groups of organic carbon compounds. These reactions are then automatically processed to give the most likely genus and species for that culture. At this time there are 526 strains/species of aerobic Gram negative bacteria, 339 for Gram positive aerobic bacteria, 361 for anaerobic bacteria, 267 for yeast, and 618 for fungi. To conduct these tests, the culture to be identified has to be a pure culture and have been identified as to whether it is Gram negative or positive, aerobic or anaerobic, or yeast or fungus. These criteria affect the type of identification that can be undertaken. There are also adaptations of the method that would allow the identification of some microbial communities. This is a laboratory-based, secondary and tertiary level identification process.

Fatty acid methyl ester (*FAME*) analysis compares differences in microbial communities based on the type and distribution of fatty acids. Another method that has been effective for the identification of microbes isolated from a water sample is the use of some of the fatty acids within the cell to identify the type of microbes present. This method uses a FAME extraction method referred to commonly as the MIDI™ system. Through libraries it is possible to identify specific cultures to appropriate genus and species identifications. To identify a specific microbial

culture the methyl esters of specific fatty acids are extracted and their levels and relationships are measured against standards. This technique involves sophisticated biochemical laboratory procedures including the use of a gas chromatograph. Using data banks it is then possible to assign a genus and species to most microbial cultures.

16S rRNA is a refined technique for looking at some of the genetic codes in microorganisms and identifying the genus, species, and strain. This method involves a very intensive series of molecular procedures but does produce a high level of precision. The libraries for bacteria currently include over 2000 base pairs some with sequencing. Identification is by the differences in the concise alignment, neighbor joining trees that are all consolidated and reporting of the results by the best fit. Some companies include both the FAME and the 16S rRNA analyses in the generation of the final analysis.

Genetic methods (*polymerase chain reaction, PCR*), in the development of more refined methods to identify microorganisms, it has been found that laboratory growth medium does not accurately reflect the true conditions found, for example, within pipelines, and therefore microbiologists have recognized that the vast majority of microbial species cannot currently be grown in the laboratory. Thus, culture-dependent approaches can underestimate the complexity of microbial communities that are actually functioning within these types of environments. Genetic methods can be used to overcome the difficulties associated with the laboratory cultivation of bacteria and provide a direct analysis of samples. In the past decade, the use of genetic techniques to detect, identify, and quantify bacteria in the medicine, food, and cosmetic industries has replaced many of the conventional microbial growth tests. These modern methods are now beginning to be employed in the energy industry for problems related to microbiologically influenced corrosion (MIC) but it is likely that genetic techniques will be the methods of choice for monitoring MIC in the future. Initial efforts to introduce the use of genetic techniques for monitoring MIC or other environmental samples have involved a type of DNA hybridization test called reverse sample genome probing. There are other hybridization-based genetic techniques including whole cell in situ fluorescent hybridization, DNA amplification followed by hybridization (dot-blot hybridization) or gel electrophoresis (denaturing gradient gel electrophoresis) that could also potentially be used to examine MIC samples. Another type of genetic test method that could be used to investigate MIC samples is based on DNA amplification using PCR. Polymerase chain reaction-based approaches include quantitative competitive PCR, quantitative real-time PCR, and reverse transcriptase PCR.

Summary, biochemical test methods. There are many modern approaches to the identification of microorganisms active within water samples and on materials that are biofouled. At this time, precision is appearing to be achieved in the sophisticated biochemistry laboratory at a significant cost. In the medical industry, such costs can well be afforded when human life and well being are directly at stake, but in the water well industry where water is still grossly undervalued, the application of such techniques may bear too high a cost to allow economical use. Of the various techniques described under the biochemically based systems it is probable that the ATP test has the most merit since it does give a direct gauge of how active and

abundant the microorganisms are within a sample, without the differentiation of the precise nature of these microbes. However, the ATP still comes with a significant laboratory analytical service charge and simply quantifies the gross levels of microbial activity. Such activity may be already recognizable for some of the symptoms present in that sample such as clouding, odors, colors, texture, and evidence of plugging or corrosion in the immediate area of the sampling. Cultural approaches play a role to determine, in a simple manner, the nature of the various microorganisms present in the biofouling community.

A.9 TESTING FOR MOLDS IN WATER WELLS

Molds are a common problem in buildings where there is high humidity. However, in water wells and groundwaters, molds would not normally be a major concern because these sites are saturated with water (molds prefer a semi-saturated environment) and oxygen is restricted (molds prefer very oxidative environments with available oxygen). It would therefore not normally be expected to find molds being a major problem in water wells unless the wells are very shallow and being fed from an aquifer where the groundwater is coming into contact with semi-saturated zones. Much of the mold coming into the water sample would be in the form of spores. A single mat of mold has the potential to release millions of spores that then move with the groundwater flow as electrically neutral bodies and tend not to get tied up in the EPS (slimes, biofilms, and encrustations) that would be growing in, and around, the bore holes.

There are many tests for molds that can be applied to water and most involve the use of agar spreadplates using selective media that encourages the growth of molds. One common selective medium in use is potato dextrose agar. Here the mold spores present in the water sample that has been streaked over the agar surface will germinate and grow to form colonies. Generally mold colonies differ from bacterial colonies in that the surfaces tend to be fuzzy due to the growth of spore bearing outgrowths (sporangia) from the mold mat (mycelium). The colors and textures of these mold colonies is usually distinctive ranging from bluish green (e.g., species of *Penicillium*) to black (e.g., species of *Mucor*, *Aspergillus*). A mycologist (specialist in molds, fungi) can often identify the genus from the colonial form of the growth and the microscopic structures in the sporangia.

Shallow groundwater that is under the direct influence of surface water, is likely to have a significant mold spore population. This is particularly the case if the surface water passes a semi-saturated redox front below the grade. Here the surface water moving down through such a front is likely to pick up, and carry, large populations of mold spores.

A.10 TESTING FOR VIRUSES IN WATER WELLS

Bioaccumulation and degradation of the virus particles within natural groundwater systems is an area of possibilities that have not been explored in

great detail. Viruses are presently a major concern since there have been cases of viruses being recovered from groundwater. In practice, there are two broad groups of viruses of interest. One group relates to bacterial viruses (called bacteriophage). These resemble nano-sized lunar landers that attach to the outer wall of the bacteria and then inject DNA into the cell. This DNA now takes over the cells function and simply reproduces the phage particles until the cell collapses. When this happens then all of the phage particles are released to now infect other bacterial cells that are vulnerable. Coliform bacteria can become infested with phage known as the coliphage and these bacterial viruses are sometimes used to indicate that there is a potential health risk associated with the water. Coliphage can be used as downstream indicators of the fact that coliform bacteria including *E. coli* was present prior to, but replaced by, the coliphage.

Animal viruses and, in particular, human viruses, can also survive in groundwater. For example in well waters, the following viruses have been identified: *rotaviruses*, *noroviruses*, *hepatitis A virus*, and *enteroviruses*. *Rotaviruses* cause severe diarrhea in young children; adults are generally immune to infection until they reach an elderly age. *Noroviruses* cause vomiting and diarrhea. Immunity to noroviruses is short-lived so all ages can be infected at any time. People will often say they had the "flu" when in fact they have had a norovirus infection. *Hepatitis A* virus infection can range from mild fever and nausea to severe liver infection with jaundice. The most severe symptoms are found in older people. *Enteroviruses* are a large group of viruses that can cause a variety of symptoms and diseases. Common symptoms include fever, diarrhea, or the common cold. In the worst case, *enteroviruses* can infect the heart, lungs, or nervous system resulting in severe long-term illness.

It is however, not clear if there are long-term public health implications from viruses are being present in groundwater at the measurable levels. When viruses are present, the level of exposure from drinking untreated water is likely to be low compared to more common ways for viruses to spread (e.g., shaking hands, exchanging money). More research is needed in this area since there are very few studies that have been performed on the occurrence of viruses in drinking water supplies.

Tests are able to detect 1 infectious virus in 50 gallons of water. To give this number some frame of reference, the U.S. Environmental Protection Agency estimates that the virus concentration at the tap is commonly from 1 virus in 130 gallons to 1 virus per 1.3 million gallons. The range in concentrations is due to the fact that different viruses have different levels of infectiousness. Chlorination provides reliable disinfection for bacteria. Available information indicates that many common viruses can be inactivated by chlorine disinfection, depending upon dosage rate and exposure time.

Since the understanding of the role and risks of viruses in groundwater is still in its infancy, there needs to be a risk analysis of the survivability of viruses in the groundwater systems. Such an evaluation of the risks would have to consider the different groups of viruses that may be entering the groundwater particularly from surface waters. Concurrently there still needs to be an evaluation of the

methodologies currently employed to detect viruses in groundwaters to determine their robustness at detecting these viruses with precision, sensitivity, and in an economic manner. It would be realistic to project the need to develop a rapid field oriented economical test for either the presence of viruses, or a suitable indicator that would relate directly to the presence of viruses in groundwater.

> A microscopic examination of the material composing these ochre-beds shows a collection of hollow tubes impregnated with iron. These are the remains of the iron-bacteria. It is evident that as other micro-organisms—or the vast majority of them—that are present do not collect the iron salts with anything like the same avidity, there must be some physiological activity or some peculiarity of structure in the iron-bacteria which enables them to bring about such a state of matters. (Ellis, D., 1919, *Iron Bacteria*. London, Methuen & Co. Ltd., 126–7.)

B BART Methodologies

B.1 INTRODUCTION

BART started its life with a frustration that there were no simple tests that could be employed in the field on water samples without the need to bring the sample back to the laboratory for testing. The BART was first conceived in 1986 and patent protected. One major difference between the BART system and other microbiological tests is that, apart from being simple to use, it does provide a greater range of environments than other systems. These environments include the creation of a redox front through the activities of the microbes in the sample. The reductive side of this front lies under the oxidative and the front moves as the oxygen in the sample is used up. Additionally, a rich source of selective chemicals is placed in the bottom of the BART tester. These chemicals diffuse up as they dissolve. This diffusion gradient forms slowly enough that it does not suddenly traumatize or harm the microbes that are being encouraged to become active through the nature of these selective chemicals. There is a ball sitting above the sample, called the floating intercedent device (or BART ball) and its position restricts the rate of oxygen penetration into the culturing fluids of the sample. This allows the redox front to be stable and slowly rise as the oxygen is consumed. In concept, the BART determines, by visible changes, the activity (growth and production of bubbles) and reactions (color changes within the tester). It is not the total cell population that the BART is determining but the level of activity of the microbes. Estimating the activity level is very important since it is the active cells that are likely to be causing the problems and not the dormant (sleeping) cells! Activity and reactions are measured by the time lapse (TL) from setting up the test to the first visible recognizable reaction or activity that is defined in the protocols for that type of BART. Time lapse can be measured in seconds, hours, and days and can occur in less than 2000 s (BOD-BART in primary effluent) to as long as 15 days [SRB-BART in a well with a deeply set anaerobic sulfate reducing bacteria (SRB) community that is not very active]. Time lapse is used to define the level of activity (or aggressivity) of the microbial community generally into four categories which are high, moderate (medium), low, and background. Very few water samples are actually sterile but most will contain at least a small number of cells that are not very active.

Another major factor is the temperature at which the BART test is incubated. There are some temperatures that many microbes really like (optimal) while other temperatures may cause the microbes to become much less active. Convenience would dictate that room temperature would be a good temperature, after all we like

22°C–24°C and so why should not microbes? Well actually, a lot of microbes grow optimally at 27°C–30°C. For this reason 28 ± 1°C is used for the majority of HAB-BART testing when precision and speed are important in getting the results. Water samples that have come from colder places such as water wells in Western Canada are commonly at temperatures of less than 15°C. For microbes accustomed to growing at these lower temperatures an optimal temperature could be 12 ± 1°C. Water from warmer waters, such as in the tropics or close to hot springs, generally will support microbes that have a higher optimal temperature, therefore 36 ± 1°C can be used. Time lapse will project a different level of activity and predicted population depending upon the incubation temperature.

It is possible to predict the active population operating within a sample on the assumption that only active cells are being included in the detection process (and dormant inactive cells excluded). Populations are expressed as predicted active cells per milliliter (or p.a.c./mL) and relate directly to the microbial community that was active in the BART tester when the sample under examination was incubated.

There are three different components that can be employed for a BART test. Of these, the BART tester is essential since the sample test is actually performed in the tester. There is also a BART reader that can be used to record the data as the BART tests are running. There are a range of readers from the V-BART-READ that will video record a series of sequences of the reaction and allow the operator to determine the TL, through to the fully functional HAB-BART readers that will declare the TL on screen once it has occurred. If the HAB-BART system is connected to a computer then HAB-BART-READ software programs can allow the real-time presentation of the graphical data with instant interpretation when the TL is declared. Definitions of the three basic components are listed below:

BART tester uses 15 mL of sample that would provide an accuracy limit at 67 cells/L where a whole liquid sample is used. If a semisolid or solid sample is used then the application may range from 10% (1.5 g) down to 1% (0.15 g). Construction involves a tube traditionally made of polystyrene and a cap made of polyurethane which is loosely screwed down to allow oxygen to enter the tester.

BART readers can employ two, six, or eight pod channels each of which can contain one BART tester. The reader contains an incubator to control the temperature which needs to be at least 5°C above ambient to achieve an acceptable variability (< 1.0°C). Reactions are detected by either shifts in sorption currently set using light pathways (daylight, red, or infra-red) having an diameter of 3 mm and radiating outwards at an angle of 30° (sorption system) with the measurement employing a photodetector; or by video recording the changes in the BART tester (V-BART-READ) uses while video recording uses daylight led lights and a universal serial bus (USB 2.0) compatible video camera with the eight channel rack.

BART software has two purposes:

1. BART-READ allows the computer to receive, save, interpret, and display activities in any of the pod testers present in an active reader (for either the HAB-BART or V-BART readers).
2. BART-SOFT allows the field operator to interpret the TLs and reactions from a tester observed when a reader is not being employed.

There is a third software program called BART QuickPop that allows the conversion of TL into population for room temperature.

B.2 SETTING UP BART TEST

BART testers come in two different formats, field and lab versions. For people wanting to work in the field away from base there is the field tester which includes an outer vial. This outer vial provides the benefits of keeping the smells created by the bacteria in the inner vial from getting out; and also being used to collect the water sample before testing. Here the outer vial can be first used to take the sample and after that is used to protect the inner tester and the operator. For people working in a laboratory there are laboratory versions of the testers. These do not have the outer vial and are more economical. The laboratory versions still generate the same reactions and activities as the field tester does but occupy less space and cost less to purchase.

Setting up a BART test involves a number of steps that are summarized in the protocols. Fundamentally 15 mL of the water sample is added to the inner test vial. When this is done the BART ball floats up and restricts oxygen entry from the headspace into the sample under test. The test begins as soon as the water sample has been added. Do not shake or disturb the test. Care needs to be taken to ensure that a redox gradient forms within the culturing fluids, made up from the sample added to the test, the chemicals diffusing up from the base, and also the oxygen diffusing downwards around the BART ball. It is important to get the tester at the temperature whether it be room temperature (22 ± 1°C) but other possible temperatures include 28 ± 1°C, 36 ± 1°C, or 12 ± 1°C depending upon the temperature of the sample when it was taken. BART testers being incubated should be kept in a location where they are not likely to be disturbed and out of any direct sunlight. Regular room lights do not influence the rate of the reactions and activities that might occur in the BART tester. Note that only 22 ± 1°C is supported by a full interpretation of TLs to population.

At regular intervals depending upon the incubation temperature, the BART testing needs to be visually examined to determine whether any reaction or activity events have occurred if no BART reader is being employed. These intervals for examination would be: room temperature—daily, 28 ± 1°C—twice a day, 36 ± 1°C—twice a day, or 12 ± 1°C—daily. When a reaction or activity occurs it can be recorded. Time lapse is calculated from the first reaction or activity that is recorded. It is taken to be the time from the start of the test to that time when the first reaction

or activity was recorded. It can be expressed in seconds, hours, or days and QuickPop does allow interpretation of any of these units into a population expressed as predicted active cells per milliliter (or p.a.c./mL). BART-SOFT can also be used to record all of the data and it will also interpret activity (aggressivity), and the nature of the bacterial community detected, as well as the population. If the BART tester is being used in a BART reader (either the video or sorption type) then the reader will detect and record the TL. If the reader is connected to a computer operating BART-READ then real-time graphs and interpretation can be generated including the prediction of the population or significant activity.

There are many levels at which many of the BART testers can be used from the field to the laboratory using the sophisticated readers and software. Each tester has its own unique features and these are addressed below. BART systems (combinations of testers, readers with appropriate software) offer a different approach to the monitoring of microbial events for diagnostic purposes and are more all embracing than the traditional cultural methods as well as being more economical, faster, and more convenient than the new generation of genomic testing that is presently being promoted.

Historically, the beginnings of the BART can be traced back to the 1880s when Winogradski discovered a unique event in which different microorganisms grew at different lateral positions down a water column in a glass measuring cylinder placed in a north facing window. This concept is still a standard part of the teaching of microbiology. The patented BART tester builds up from the Winogradski findings through the discovery that these growths were, in part, a result of the development of opposing selective nutrient gradients rising with an oxidation front descending. Different communities of microbes from within the sample find different sites within the testers culturing fluids to become active and grow. Today the BART testers are used mostly in the water, chemical, oil, and gas industries. The major differences between the BART system and classical microbiological techniques are:

BART tests encourage all microorganisms active in the sample to be able to grow in one or more of the various dynamic environments within the tester. Classical microbiological techniques differentiate at the species/taxon level with the exclusion of many of the organisms in the sample because they do not provide such a variety of environments.

BART technologies examine the activity levels of the cells while classical techniques (commonly based on agar media) automatically become highly selective but usually involve a limited range of species and generally do not have the ability to culturally detect the whole of active microbial communities.

Full BART systems include sequential colorimetric sensing of the testers in a manner that generates an interpretable data stream which can be transmitted to distant locations for confirmatory and archiving purposes. For the V reader system that can record the activities and reactions of a variety of BART testers at the same time, the data are locked in as read-only files that may be viewed for analysis but not altered. Such data protection allows potential acceptance as legal evidence if the chain of custody for the sample is certified and secure.

Traditional scientific thinking commonly takes microbiology to the species level without consideration of community interactions. BART testers focus on microbial communities at large rather than single species. Little is known of the roles (protective/infective) of these communities in warm blooded animals (including humans). This traditional focus at the species level has probably neglected the powerful roles that microbial communities can play in the control of, and recovery from, significant infections in water wells or even human beings! It could also be argued that it could be the microbial communities that play a major role in the transmission of virus particles between infection sites in different hosts. This whole area of microbiology has not been explored and the BART systems provide a simple method to detect these communities.

In the evolution of the BART system since 1986, there has not been a major effort to enter the health and food industries with two exceptions:

1. Urinary tract infections where a combination of salinity and alkalinity spontaneously generated visible colloidal flocs in less than 900 s.
2. Microbial loadings in cow's milk were determined to be practicable using the HAB-BART system but employing a reflectometric light sensing system to detect reductive activities. In the latter case, TLs commonly ranged from 18,000 to 65,000 s.

This illustrates that the BART testers have a potential to fulfill the role as the prime first-level testing system for the detection of significant microbial activity.

In technologist time directly committed to the testing, the BART system generally takes less than 3 min to set up and then the time to check for reactions and activities. Data can then be stored for future reference if required. For water well operators, a timed chain of sampling and BART testing can be used to determine when a water well is beginning to fail due to biofouling and also whether a treatment that has been applied to the well has been successful.

BART systems therefore offer a different approach to the monitoring of microbial events for diagnostic purposes and are far more all embracing than the traditional cultural methods as well as being more economical, faster, and convenient than the new generation of genomic testing that is presently being promoted.

B.3 RED CAPPED IRON-RELATED BACTERIA—IRB-BART

IRB-BART tests are more complex because of the numbers of reactions that can occur in what can be construed as sometimes formless manners. Iron-related bacteria (IRB) is the name applied to that group of bacteria that, for various reasons, accumulate iron within the colloidal or colonial growths in a manner that significantly exceeds immediate metabolic requirements for iron. Commonly this accumulation is in the form of crystallized ferric iron as oxides, hydroxides, and sometimes carbonates. Generally this type of growth takes on the color of the ferric

accumulates and appears to be the color of rust. Hence, the common name applied is iron bacteria to reflect the fact that these bacteria accumulate iron. The term "IRB" was developed to reflect, in a more qualitative manner, the various forms of bacteria that can be associated not just with the accumulation of ferric iron but also the dissolution of iron under more reductive conditions. The definition of IRB is therefore:

> Incorporates all bacteria that are able to accumulate excessive amounts of iron in any form within the environmental matrix within which they function.

This brief definition encompasses a number of major bacterial groups that have been commonly recognized but not necessarily associated within a common grouping. These groups are described below separately and their role within the iron-related bacterial group defined primarily in relation to the oxidation–reduction potential (ORP) of the environment within which they are active. Generally the different groups are active in specific parts of the ORP gradient and can attach to surfaces through the generation of biofilms, encrustations, nodules, or some other form of growth. These are described below:

Iron oxidizing bacteria (*ferrous oxidizing bacteria*) is the name given to those bacteria that are able to accumulate ferric forms of oxide and hydroxide within the growing biomass. This accumulation appears commonly to be ongoing until the Fe content reaches between 40 and 95% of the dried weight. These events commonly occur at the redox front between oxidative and reductive conditions usually with ORP values of between +10 and −50 mV. These bacteria are often involved in the biological formation of iron ochre.

Iron reducing bacteria (*ferric reducing bacteria FRB*) is that group of bacteria which, under various reductive (anaerobic oxygen-free) conditions, reduce ferric forms of iron to the more soluble ferrous that may then move by diffusive and biocolloidal processes out of the immediate environment where they were created. Iron-reducing bacteria are therefore most commonly found under more reductive conditions where the ORP values are from 0 to −150 mV.

Sheathed iron bacteria are bacteria which possess the ability to live for at least a part of their activity cycle within a slime-like sheath onto which ferric iron may, or may not, be accumulated. These sheaths usually are formed in the upper more oxidative part of the biomass and frequently the sheaths will extend out into the water flow. These bacteria are able to move into (for protection and growth) and out of (for colonization) the sheaths in accordance with the stage of the life cycle these bacteria are passing through. Generally the ORP values range from +5 to +150 mV in order to get these sheathed bacteria to become a significant part of the biomass.

Ribbon iron bacteria (*RIB*) are some bacteria that have the ability to extrude slime-like ribbons out of the cell that are rich in ferric forms of iron. Usually only one ribbon is generated for each cell and it is thought that the energy release from the oxidation of ferrous to ferric forms of iron provides a significant part of the total energy requirement. Generally these bacteria (dominated by the genus *Gallionella*) grow on the surface of the growing biomass with the ribbons extending outwards into the free water flow. It is thought that these ribbons act using the Archimedean screw principles to move nutrients from the water down to the cell. When mature, these ribbons break off and move into the water flow where their presence is easy to observe microscopically. It should be remembered that the presence of these ribbons in a water sample does not mean that cells of *Gallionella* are present, it simply means that some *Gallionella* growths occurred upstream and that growth was shedding ribbons. Commonly species of *Gallionella* grow under oxidative conditions ($> +50$ mV) over very sharp redox fronts created within the biomass that allows ferrous iron to become available to the cells. This ferrous iron could have originated in the water flowing over the biomass in the ferric form that was taken up and reduced to the ferrous form or it could have been ferrous iron diffusing out from the deeper reductive parts of the biomass.

B.3.1 Qualitative Interpretation of IRB Reaction Patterns

IRB-BART reaction patterns follow in a sequence that indicates many of the characteristics of the IRB within the sample being tested. There are two major pathways for the sequence of reactions observable in the IRB-BART tester with a number of different combinations. There is a sequence in the occurrences of the reactions which is dependent mainly upon the microbes that are active within the sample now being tested. The term "microbes" is used here because fungi and possibly protozoa and algae may also influence the formation of a particular sequence of reaction. The common sequences for the reaction to occur are divided into four chronological sequences. This sequence of possible reactions can begin with the development of a white base (WB) reaction which forms as a white deposit of carbonates in the base of the tester. Usually this occurs within 12 h of the test starting (sequence one) and has traditionally not been recognized as a reaction in the interpretation of the IRB in the sample but it is important in the determination for the treatment of biofouling in wells, cooling towers, and water treatment systems. The presence of the WB does indicate that carbonates are capable of being accumulated and that any regeneration treatment should take into account the need to disrupt carbonate-rich encrustations.

The first major diagnostic sequence (Table B.1, reaction patterns and sequences for the IRB-BART tester) that occurs usually in 1–5 days is the generation of either a cloudy (CL) or foam (FO) reaction as sequence two. Cloudy occurs more commonly when the sample comes from an oxidative region or at the redox front (where there is an interface between oxidative and reductive conditions). Both the FO and CL

TABLE B.1
Reaction Pattern Sequences in the IRB-BART

Sequence	Reaction Pattern	Comments
1	White base (WB)	Occurs more commonly in slightly alkaline samples
2	Clouding (CL)	Indicates bacterial activity is occurring in the culturing fluids of the tester
	Foam formation	Reductive fermentative activity leads to the generation of gases including some of carbon dioxide, hydrogen, methane, and nitrogen
3	Brown clouding	Iron is being accumulated within the biocolloids created by some ferrous oxidizing bacteria (FOB)
	Brown ring	FOB, ribbon iron bacteria, and sheathed iron bacteria are all capable of becoming significant when a ferric-rich slime ring forms
	Brown gel	Some enteric bacteria (species of *Enterobacter* and *Klebsiella*) generate a dense ferric-rich gel that settles in the tester
	Red clouding	Some enteric bacteria (including species of *Serratia*) will cause a red color to be generated in the clouded growth
	Green clouding	This starts as a weak CL reaction that slowly turns a light shade of green that may turn darker and cloudier over time. This event occurs when *Pseudomonas* species are very active
4	Black liquid (BL)	Walls and base of the tester get coated with black materials (possibly dominated by iron carbonates). This happens commonly when both enteric and pseudomonad bacteria are active

Note: Sequence refers to the order in which the reactions are likely to occur over time with one being the most likely to occur first and four would be the final reaction. This order is dependent upon microorganisms being present and active enough to cause those specific reactions. WB is not a recognized bacterial reaction but can be used to design chemical treatments to be effective against carbonates.

reactions can occur together meaning that the microbes in the sample are mixtures of cells capable of being active both oxidatively (through respiration) and reductively (through fermentation). If the FO precedes the CL then the sample is more dominated by fermentors whereas if the CL precedes the FO then it is dominated by "respirers." Reactions found in sequence three tend to occur in an order reflecting the dominance of those particular bacteria. Sequence four is a terminal reaction which occurs only when there are significant populations of enteric and pseudomonad bacteria present.

The location of any given reaction type within the IRB-BART tester gives information about the active bacterial community present in the sample. It also can be used to determine the ORP state where the sample was taken. Reactions can be interpreted using BART-SOFT but these can be summarized below:

1. Cloudy—"heterotrophic bacteria are present."
2. Foam—"anaerobic bacterial activity detected."
3. Brown clouding—"IRB are active."
4. Brown gel—"dense slime forming enteric bacteria located."
5. Brown ring—"very aerobic slime forming IRB observed."
6. Green clouding—"heterotrophic pseudomonad-type bacteria appear dominant."
7. Red clouding—"enteric bacteria are very common."
8. Blackened liquid—"there is also a dominant mixture of pseudomonad and enteric bacterial species that could form a health risk then it is advised to conduct a coliform test."

The WB reaction, where carbonates have been formed, is not used to identify bacteria but it does indicate that there is a strong risk that carbonates would have been formed as a part of the biofouling event. If this occurs then the regeneration treatment should employ an acid phase to destroy these carbonates.

The term "IRB" implies strongly that all of the microbes cultured using the IRB-BART tester are most likely to be involved in some form of excessive manipulation of iron beyond the basic needs (Table B.2). For microorganisms to be cultured and recognized within the IRB-BART tester there are a number of constraints that reduce the probability of non-IRB growing within these culturing fluids. Within the nutrient pellet dispensed in the tester are chemicals that will restrict the ability of many bacteria to grow. Through the application of a crystallized pellet for the delivery of these selective nutrients, there is a gradual concentration increase of these selective chemicals as they diffuse up through the culturing fluids. This then forms a vertically moving diffusion front that further limits the types of bacterial activity to the IRB.

For the IRB-BART, claims relate to the fact that the IRB-BART generates, when charged with a water sample, a sufficient diversity of environments that will encourage the determination of observable activities of the IRB within the water sample being tested. From experiences to date the IRB-BART tester appears to be superior to any other field-applicable testing system due to the broad scope of IRB that can be recovered using this tester.

These claims presented would be subject to the following limitations:

1. The limits of detection for the IRB in a given water sample would be 67 cells/L.
2. Any water sample taken for testing using the IRB-BART tester would have to be collected following the protocols established for the collection of a water sample for microbiological analysis. Transportation and storage of the sample should similarly follow the standard guidelines practiced for sample handling prior to the initiation of microbiological examination. These should include hygienic aseptic handling, the use of sterile sample containers, and minimizing the sample storage time to less than 4 h at room temperature or 24 h when cooled to refrigeration temperatures.

TABLE B.2
Chemical Factors Used to Define the IRB

Ingredient	Restrictive Function
Ferric iron	Ferric iron is provided in sufficiently high concentrations to be inhibitory to bacteria that do not have a high tolerance. It has been found that the ferric form will be commonly reduced to the ferrous form by many of the IRB to allow the iron cycle to occur
Citrate	By experiment it has been shown that most of the IRB are able to most commonly utilize citrate as carbon source. Citrate therefore acts as a functional restrictor for the many heterotrophic bacteria that cannot use this carbon source
Nitrate	This chemical provides an alternate electron acceptor to oxygen and many of the IRB are able to "respire" using this nitrate. Therefore it provides an improved selectivity for the growth of IRB
Dipotassium hydrogen phosphate	IRB commonly have the ability to store phosphate primarily as polyphosphates. The use of a high concentration of phosphate restricts the range of bacteria able to grow to those that are phosphate tolerant

3. The IRB-BART can be used for both field- and laboratory-based investigations and generate similar data with respect to TL and reaction patterns where a sample is split and incubated under similar conditions in field and laboratory settings.
4. While the IRB-BART technology commonly operates at ambient room temperatures there is the ability for the testers to be used at incubation temperatures ranging from +1 to +30°C under exceptional circumstances. Incubation of the IRB-BART at temperatures higher than 30°C is not recommended because of distortions to reactions that can occur.

B.4 BLACK CAPPED SRB—SRB-BART

The SRB-BART tester uses a broad spectrum Postgate medium commonly recognized as being able to detect a range of SRB. Functionally, this is achieved by the establishment of the redox gradient caused by the BART ball to restrict oxygen entry downwards into the sample (culturing fluids). Hence, the lower regions of the tester become reductive while the upper regions remain oxidative. These conditions generate one of two SRB reaction patterns. Before adopting the test method, comparisons were undertaken with the standard tests and it was found that the SRB tester had a greater sensitivity, was easier to use, and provided more reliable data than the industry standard ("nail in the bottle" test) (Table B.3).

The tester uses a selective medium that encourages the significant growth of SRB. Chemicals employed in the SRB-BART tester are listed in Table B.4. Sulfate reducing bacteria is the name applied to that group of bacteria that are able to reduce sulfate with the gaseous production of hydrogen sulfide. This gas now reacts with

TABLE B.3
SRB Reaction Patterns, Diagnostic Comments

Reaction, RX	Description	Comments
Black top	Black specks or dark gray film on the lower side of the BART ball	SRB are growing in association within bacterial consortia dominated by aerobic bacteria that function under oxidative conditions
Black base	Radial blackening of the cone in the base of the tester that continues until the whole base is black	SRB are growing under reductive conditions in a covert manner without necessarily growing in association with other bacterial species

iron to make black iron sulfide, generates black slimes, and starts corrosive processes. Sulfate reducing bacteria are generally thought to be a major cause of corrosion of iron fabricated industrial equipment and facilities, steel pipelines, storage tanks, and even in concrete. Sulfate reducing bacteria are generally considered to include:

> All bacteria that reduce sulfate or sulfur to hydrogen sulfide, they usually are active under oxygen-free (i.e., reductive, anaerobic) conditions and use fatty acids (particularly acetate) as the main source of organic carbon.

TABLE B.4
Selective Chemical Ingredients Use in the SRB-BART Tester

Chemical	Comment on Selection
Sulfate	This is the chemical that become reduced by the sulfate reducing bacteria (SRB) to hydrogen sulfide
Ferrous iron	Once hydrogen sulfide has been generated by the SRB it reacts with the ferrous iron to produce black sulfide that becomes the diagnostic feature for as SRB-BART-positive test
Acetate	Acetate is a major carbon substrate used by SRB for growth and metabolism. In the SRB-BART tester the methane producing bacteria may compete with the SRB causing significant gas production. However the conditions in the tester favor the SRB rather than the methane producers
Anoxic blocker	This chemical restricts the diffusion of oxygen down around the ball. This allows reductive conditions to be set up more quickly and encourages the growth of the black top (BT) reaction

This brief definition encompasses a number of major SRB that have been commonly recognized as using either a different sulfur-based substrate or the ability to produce spores.

Sulfate reducing bacteria (SRB) are the dominant SRB with the dominant genus in this group being *Desulfovibrio*. These bacteria can be found in two locations. First, they are commonly found at the redox front between oxidative and reductive conditions usually with ORP values of between +10 and −50 mV but also where there is also extensive heterotrophic bacterial activity and the tend to live deeper down in the biofilms. Second, some SRB will grow in much more reductive environments commonly between ORP values are from −20 to −150 mV. *Desulfovibrio* is the most common genus associated with corrosive processes.

There are only two reactions of significance for the SRB-BART. In the black top (BT) reaction, a positive can be recognized by the generation of 1–2 mm small black specks or the gray color developing on the lower part of the BART ball. For the BB (black base) reaction, a positive is recognized when there is a jet black region formed in the cone at the base of the tester (defined in Table B.3, reaction patterns for the SRB-BART tester). To view this, lift the tester and examine the cone-shaped base for blackening. Sometimes small regions may become blackened fairly quickly, usually near the center. These should not be considered positive. Only once the blackening has spread to half the area of the base cone and it is considered as a positive. These BB deposits will continue to gradually grow and cover the whole base cone and may even climb 2–4 mm up the side walls of the tester. It should be noted that there used to be a third reaction black all (BA) in which the black sulfide reactions were observed both at the top and the bottom of the tester at the same time. This can occur if the tester is visually examined daily but where this is observed, the reaction should be considered a BB since BA is no longer recognized. If the V-BART reader is used then the precise time that the BB and BT reaction occurred would be determinable. Usually either a BT or a BB reaction will advance to the BA interpretation is based upon which reaction occurs first. Black all is no longer an acceptable reaction and in the event that a BA reaction does occur without the prior observation of a BT or BB reaction then the reaction defaults as a BB reaction.

These two reactions signify that a different community of bacteria of SRB are present and causing either the BT or BB reaction. Both can involve species of the *Desulfovibrio* and *Desulfotomaculum* (this latter genus within the SRB is actually able produce spores that can survive higher temperatures than the cell).

B.5 LIME GREEN CAPPED SLIME-FORMING BACTERIA—SLYM-BART

Slime-forming bacteria (SLYM) is the name given to bacteria that are able to produce copious amounts of slime without necessarily having to accumulate any iron. These slime-like growths are therefore not dominated by the yellows, reds, and browns commonly seen where IRB are present. Some of the IRB also produce slime but it is sometimes denser and has more texture due to the accumulation of various forms of insoluble iron. SLYM-BART bacteria can also function under different

reduction–oxidation (REDOX) conditions but generally produce the thickest slime formations under aerobic (oxidative) conditions. These can develop in the SLYM-BART™ as slime rings (SRs) growing around the BART ball. Slime growth can also be seen as CL (fluffy or tight plate-like structures) or as gel-like growths which may be localized or occur generally within the body of water sample being tested. Commonly, gel-like slime growths form from the bottom up in the test vials. One common check for these types of growth is to tilt the BART™ gently and see that the CL gel-like growths retain their structure and move with the tube's motion. Almost all of the SLYM can produce copious amounts of slime that can contribute to plugging, loss in efficiency of heat exchangers, clouding, bad taste, and odor problems. This is one of the most sensitive BART™ tests. A positive involves a CL reaction in the tester often with thick gel-like rings around the ball. A negative test remains clear. A vast majority of bacteria can produce slime-like growths. The slime is actually formed by a variety of extracellular polymers that are long thread-like stringy molecules. These extracellular polymeric substances (EPS) literally coat the cells into a common slime-mass within which large volumes of water become clustered and bound. Often 95%–99% of the volume of slime is actually water. Some bacteria produce an EPS that remains tightly bound to the individual cell. These are called capsules. Other bacteria generate such a copious amount of EPS that it envelops whole masses of cells within a common slime.

The role of the slime appears to be protective. If environmental conditions are harsh (e.g., due to shortage of nutrients), the slime layers tend to get thicker. Not only does the slime act as a protectant to the resident bacteria but it also acts as a bio-sponge by accumulating many chemicals that could form either a nutrient base, or be toxic to the cells. Extracellular polymeric substances may be produced by enzymatic activity (e.g., dextran sucrase, or levan sucrase) on carbohydrates. In addition, EPS may be synthesized within the bacterial cells and released to form an enveloping slime. Reaction patterns for the SLYM-BART™ tester are described below:

DS—dense slime this reaction may not be obvious and may require the observer to gently rotate the SLYM-BART™ tester at which time the slimy deposits will swirl up possibly in the form of a twisting slime. This swirl can reach 40 mm up into the culturing fluids, or it may rise up as globular gel-like masses that settle fairly quickly. Once the swirl has settled down, the liquid may become clear again. In the latter case, care should be taken to confirm that the artifact is biological (ill-defined edge, mucoid, globular) rather than chemical (defined edge, crystalline, often white or translucent). Generally, these DS growths are beige, white, or yellowish-orange in color. This reaction is defined as including dense SLYM producing copious EPS, with facultative anaerobes dominating.

CP—cloudy plates layering, when there are populations of aerobic bacteria, the initial growth may be at the redox front that commonly forms above the yellowish-brown diffusion front. This growth usually takes the form of lateral or "puffy" clouding which is most commonly gray in color. Often the lateral clouds may be disc-like in shape (plates) and relatively thin (1–2 mm). It should be noted that if the observer tips the BART™ slightly,

the clouds or plates often move to maintain position within the tube. The edges of the plates are distinct while the edges of the "puffy" forms of layering are indistinct. These formations are most commonly observed 15–30 mm beneath the fill line. Cloudy formations will tend to extend to cause an overall cloudiness of the liquid medium (CL). These plates sometimes appear to divide (multiple plating) before coalescing into a CL liquid medium.

SR—slime ring occurs usually 2–5 mm in width, forms on the upper side of the BART ball. Appearance is commonly mucoid and may be white, beige, yellow, orange, or violet in color that commonly get more intense over time on the upper edge.

CL—cloudy growth solution is very CL and there may sometimes be a poorly defined slime growth around the ball. Sometimes a glowing may be noticed in at least a part of the top 18 mm of the liquid medium. This glowing is due to the generation of ultraviolet (UV) fluorescent pigments by some species of *Pseudomonas*. The common pigments doing this are a pale blue (PB) or a greenish yellow (GY) color. Note that this glowing may not be readily observable unless a UV light is used. The occurrence of the glowing with a UV light means that there is a probability of potentially pathogenic species of *Pseudomonas* and confirmatory testing is recommended.

BL—blackened liquid is commonly a secondary or a tertiary reaction rather than an initial one. It is recognized as a clear, often colorless, solution that is surrounded by large blackened zones in the basal cone and up the walls of the test vial. The BL often parallels the BL reaction in the IRB when the two BARTs™ are used together to test the same water sample.

TH—thread-like strands are when, on some occasions, the slime forms into threads that form web-like patterns in the liquid medium. Sometimes these threads interconnect from the BART ball to the floor of the tester.

GY—greenish yellow glow in UV usually follows the CL reaction and a GY glow forms around the BART ball. This glow appears after 2–4 days and may extend half way down the tester and last for as long as 7 days. It can only be easily seen using a UV light. This GY reaction indicates the possible presence of *Pseudomonas fluorescens*.

PB—pale blue glow in UV, of the above reactions, usually follows the CL reaction and is a very distinctive PB color that occurs after 1 or 2 days around the BART ball and remains commonly for less than 2 days. This PB reaction indicates the possible presence of *Pseudomonas aeruginosa*.

B.6 DARK BLUE CAPPED HETEROTROPHIC BACTERIA—HAB-BART

Heterotrophic bacteria are defined as a group by their ability to exploit and biodegrade organic materials as the main source of energy, nitrogen, and

phosphorus. As a group they vary in requirements from species to species but collectively as a community possess the resources to utilize carbohydrates, lipids, proteins, and even some of the hydrocarbons such as methane. For this reason, these bacteria often dominate in conditions where there are spills such as solvents or hydrocarbons and they play major roles in degrading even toxic organic materials. The culture medium used for the HAB-BART is a crystallized culture medium rich in a diverse range of carbohydrates and proteins.

During the development of the HAB-BART tester, it was found that the up (UP) reaction tends to occur when there is a dominance of heterotrophic bacteria growing primarily aerobically. Such aerobic events are likely to occur immediately downstream from major sites of organic pollution where there is oxidative microbial activity. Dead organics in oxygen stressed conditions, by contrast, appear to occur in waters dominated by facultative and strictly anaerobic heterotrophic bacteria that are active under more reductive conditions. Here, the water sampled would be on the reductive side of any redox front where the heterotrophic bacterial activity can be expected to focus. In summary, a down (DO) reaction may imply that the biofouling is from the sample site where oxygen is absent while an UP reaction would imply that major oxidative activity is occurring in the sample where oxygen would have been present in the water (for a UP reaction to have occurred). With the DO reaction, the activity associated with the water sample would be anaerobic since it would be occurring under reductive oxygen-limited conditions. Such activities tend to generate smaller molecules as organic daughter products such as the short chain fatty acids that would then move downstream to finally breakdown when conditions become oxidative as the water crosses the redox front. Here there would be magnification of the biological activity that would then often be associable with biofouling.

One unique feature of the HAB-BART tester is that in addition to a specific enriched mineral and nutrient medium, and methylene blue (MB) is also added as a redox indicator. This addition allows respiration rates to be monitored through the TL to the initiation of bleaching (when the MB changes from a blue color to a clear state). While free oxygen remains in the culturing fluids then the MB dye in the liquid medium remains blue. However, as soon as all of the oxygen has been consumed by bacterial respiratory activity then the MB shifts to a colorless form. In other words, in the HAB-BART test, when the liquid medium turns from blue to a colorless form then the heterotrophic aerobic bacteria have been sufficiently aggressive to have "respired off" all of the oxygen from that site. Here, the rate of bleaching action is correlated with the population of heterotrophic bacteria in the sample. It should be noted that the dried MB is present in the cap of each HAB-BART tester and is dissolved into the liquid sample by inverting the HAB-BART for 30 s followed by a wrist action inversion three times. During this process, the BART ball bounces up and down the test vial a total of six times. This allows the head space air time to saturate the turbulent culturing fluid sample with oxygen to saturation.

Methylene blue (MB) is a basic dye that can bind readily to the negatively charged microbial cells. Traditionally, this dye has been used to stain microbial cells. The important property of MB dye is that it changes from its original blue

color in the oxidized state, to clear in the reduced state depending upon the amount of available oxygen. When MB is added to a liquid medium with respirable organic concentration, electrons are transferred to the dye molecule causing it to become reduced and the blue (oxidation) color disappears. The rate at which this happens is dependent upon the rate of the microbiological respiratory activity and it is this feature that allows the operator to determine the size of the active heterotrophic bacterial population.

With the UP reaction, the MB solution bleaches (decolorizes) from the bottom up. The bleached zone underneath may appear to be either clear or a clouded yellow. In the latter case, this is because the medium tends to have a light to medium yellow color. Rarely does the bleaching extend upwards beyond the equator of the BART ball so that a blue ring will normally remain around the ball with a depth of 1–5 mm below the surface for some time after the completion of the test.

With the DO reaction, the bleaching usually forms just below the BART ball in a zone 32–42 mm above the base of the tester. Unlike the UP reaction, the DO forms as a series of swirling unstable decolorized regions within the medium that then stabilizes into a clear colorless lateral zone with a front that then moves down the liquid column.

In the HAB-BART system reader there are two lateral red light pathways placed at 14 and 29 mm from the base. These lights detect when there is a change in sorption using a light pathway width of 3 mm. The recognized change in sorption units (s.u.) is from greater than 210 s.u. down to lower values than 162 s.u. On occasions, the s.u. drops to values as low as 8 s.u. or lower if there has been activity without the development of a CL biomass.

Methylene blue (MB) does have an impact on the heterotrophic bacterial activity. In a series of trials between the medium with and without MB it was found that there was a consistent difference. The controls in three trials had a TL of 1.62 ± 0.51 days while the MB (in the HAB-BART) caused a delay giving TL of 2.47 ± 0.77 days. This difference of 0.85 days, with an increased variability (of an additional ± 0.26 days), may be attributable to the MB having an inhibitory effect on the active bacterial population. For the control, the method for detecting activity was visual (formation of turbidity). In the HAB-BART, the BART reader was used to directly detect the changes in sorption as the MB was reduced. Given that the occurrence of turbidity almost always follows the development of reductive conditions (i.e., MB reduction in a HAB-BART) the probability is that MB does have an inhibitory effect on the heterotrophic bacteria that causes a prolongation of the TL. This effect would appear to be transitory since in all cases observed there was subsequent activity with the HAB-BART. It is therefore claimed that the MB does act as a transitory inhibitor to the activity of heterotrophic bacteria. This impact consistently results in an extension of the TL by an average of a 52% increase.

It should be noted that higher numbers of cells than 1 cell per 15 mL (or 67 cells/L) are needed to create a positive TL that would relate to the number of cells that did become active within the incubating tester conditions during the test period. This therefore forms the theoretical premise for the claims that the length of the TL (in seconds) is inversely related to the number of active cells within the sample being tested.

B.7 GRAY CAPPED DENITRIFYING BACTERIA—DN-BART

DN is short for denitrification. This activity is extremely important not only in environmental but also in geochemical terms. The reason for this is that essentially all of the atmospheric nitrogen (N_2) has been derived from the process of denitrification which is driven by the denitrifying bacteria. It is therefore an extremely important stage in the nitrogen cycle on the surface and immediate subsurface of planet Earth. There is a distinctive cycle in which nitrogen from the atmosphere is fixed, cycles through the biomass, is oxidized to nitrate by nitrification (see N-BART) and reduced back to nitrogen gas by denitrification which is controlled by the denitrifying bacteria. The denitrifying bacteria are therefore an important indicator group for the decomposition of waste organic nitrogenous materials. These denitrifiers reduce nitrate through to nitrite and some continue the nitrification on down to gaseous dinitrogen (complete denitrification). In waters, the presence of an aggressive population of denitrifiers can be taken to indicate that there are significant amounts of nitrate in the water. Such waters are most likely anaerobic (free of oxygen) and relatively rich in organic matter. When the DN-BART detects denitrifying bacteria in the sample a reaction occurs in the form of a ring of FO around the BART ball. This is called the FO reaction and is generating nitrogen gas that collects temporarily as FO around the BART ball before dissipating (usually after 24 h).

A common use for the presence of aggressive denitrifying bacteria in waters is that these bacteria signal the later stages in the degradation of nitrogen-rich sewage and septic wastewater. Aggressive presence of denitrifiers in water can therefore be used to indicate that there is a potential for the groundwater to have been polluted by nitrogen-rich organics from such sources as compromised septic tanks, sewage systems, industrial and hazardous waste sites. It is recommended that, where a high aggressivity is determined, the water should be subjected to further evaluation as a hygiene risk through a subsequent determination for the presence of coliform bacteria. In soils, the presence of an aggressive denitrifying bacterial population may be taken to indicate that the denitrification part of the soil nitrogen cycle is functional. It would be expected that there would be a greater potential of denitrifying bacteria in groundwater under the direct influence of surface waters where the water moves across the redox front and contains a significant concentration of inorganic and/or organic nitrogen.

This DN bacterial group actually reduces nitrates under anaerobic conditions to dinitrogen gas. This activity is significant since nitrates in water are a serious health concern particularly for babies and the presence of DN bacteria would reduce this risk under reductive conditions. In this test, this gas forms a FO from dinitrogen gas bubbles that usually collect around the ball within 3 days and last for about 1 day. The presence of this FO by the end of day three is taken to be an indication of an aggressive population of denitrifying bacteria. The absence of FO, regardless of any clouding of the water, indicates that the test is negative for the detection of denitrifying bacteria. This test is applicable to any water where there is likely to be potential septic or organic contamination. The presence of denitrifiers in water

would indicate a potential health risk due to either septic wastes or nitrates in the water.

This test detects bacteria that can reduce nitrate (NO_3) to dinitrogen gas (N_2) by the observation of gassing which occurs when the nitrate has been completely denitrified.

While nitrite is an intermediate in the denitrification of nitrates (with dinitrogen gas being the terminal product) it does not remain resident for a significant period of time. Nitrite was therefore discounted as a suitable indicator method in favor of gas production.

B.8 WHITE CAPPED NITRIFYING BACTERIA—N-BART

The nitrifying bacteria are an important indicator group for the recycling of organic nitrogenous materials from ammonium (the end point for the decomposition of proteins) to the production of nitrates. In waters, the presence of an aggressive population if nitrifiers are taken to indicate that there is a potential for significant amounts of nitrate being generated in waters which are aerobic (rich in oxygen). Nitrates in water are a cause of concern because of the potential health risk particularly to infants who have not yet developed a tolerance to nitrates. In soils, nitrification is considered to be a very significant and useful function in the recycling of nitrogen through the soil. Nitrate is a highly mobile ion in the soil and will move (diffuse) relatively quickly while ammonium remains relatively "locked" in the soil.

In some agronomic practices, nitrification inhibitors have been used to reduce the "losses" of ammonium to nitrate. A common use for the presence of active nitrifying bacteria in waters is that these bacteria signal the latter stages in the aerobic degradation of nitrogen-rich organic materials. Aggressive presence of nitrifying bacteria in water can be used to indicate that there is a potential for the water to have been polluted by nitrogen-rich organics from such sources as compromised septic tanks, sewage systems, industrial and hazardous waste sites and is undergoing an aerobic form of degradation. Nitrification and denitrification are essentially parallel processes that function in reverse sequence of each other. It is recommended that, where a high aggressivity is determined, waters should be subjected to further evaluation as a hygiene risk through a subsequent determination for the presence of nitrates. In soils, the presence of an aggressive nitrifying bacterial population may be taken to indicate that the nitrification part of the soil nitrogen cycle is functional. Nitrification is fundamentally an aerobic process in which the ammonium is oxidatively converted to nitrate via nitrite.

Nitrite can also be produced by the denitrification of nitrate and this can also be oxidized back to nitrate. There are two steps to nitrification process:

$$\underset{\text{Ammonium}}{NH_4} \xrightarrow{(1)} \underset{\text{Nitrite}}{NO_2} \xrightarrow{(2)} \underset{\text{Nitrate}}{NO_3}$$

Nitrification serves as the major route by which ammonium is aerobically oxidized to nitrate. Nitrifying bacteria are divided into groups:

Group 1 -step (1) only—Nitrosofiers—*Nitrosomonas.*
Group 2 -step (2) only—Nitrifiers—*Nitrobacter.*

The contradictory relationship between the nitrifying and the denitrifying bacteria is a problem in the testing of natural samples since these two groups can both be either producing or utilizing nitrate respectively. In developing a test system for the nitrifying bacteria in natural samples, the terminal product (nitrate) may not be recoverable with any certainty because of the intrinsic activities of the denitrifying bacteria which are also likely to be present and active in the sample. It is because of this difficulty that the N-BART tester is restricted to detecting the nitrosofiers (group 1) that generate nitrite. This nitrite will be oxidized to nitrate by the nitrifiers only to reappear when reduced back to nitrite by any intrinsic denitrification occurring in the sample.

This test is unusual in that the presence of nitrifying bacteria is detected by the presence if nitrite in the test vial after a standard incubation period of 5 days. Nitrification involves the oxidation of ammonium to nitrate via nitrite. Unfortunately, in natural samples, there are commonly denitrifying bacteria present in the water and these reduce the nitrate back to nitrite. If denitrification is completed, this nitrite may be reduced further to dinitrogen gas (under anaerobic conditions). That is why this test is laid upon its side with three balls to provide a moistened highly aerobic upper surface where nitrification is most likely to occur. The reagent administered in the reaction cap detects nitrite specifically by a pink-red color reaction. This test is interpreted by the amount of pink-red coloration generated, and the location of this color:

PP—Pink-red color on roughly half the ball, solution clear or yellow (Reaction 1).
RP—All balls are reddened, solution may be pale pink (Reaction 2).
DR—Balls and the solution is reddened (Reaction 3).

This test is different to the other BART™ tests in that a chemical reagent is added to detect the product (nitrite) after a standard incubation period. The typical reactions are described below:

PP—Partial Pink on the Balls. Clear solution but a pink reaction may be generated on the BART ball indicating that nitrification has just begun and the nitrite detected is in the biofilm on the balls.
RP—Red Deposits and Pink Solution. Reaction causes a light pink solution with red deposits all over the three BART balls. Nitrite is now present in solution as well as in the biofilms on the BART balls.
DR—Dark Red Deposits and Solution. Reaction causes dark red solution with heavy red deposits on BART ball. High concentrations of nitrite have been detected indicating an aggressive level of nitrification has occurred in the test period.

B.9 DARK GREEN CAPPED ALGAE—ALGE-BART

Algae are simple plants having a simple cell form not differentiated into tissues. As a result algae are normally small, growing either attached to surfaces (sessile) or floating freely in the water (planktonic). Water samples are more likely to contain the planktonic type of algae unless the attached algae have been released into the water through some form of disturbance that could have been caused by changes in the patterns of water flow or physical agitation. Algae have a tendency to stratify within the sample it would also be important to agitate the sample before setting up the ALGE-BART (Table B.5). Algae are not commonly detected in groundwater unless there is some direct influence from surface waters where the algae are able to grow. Occasionally algae are reported in water wells (particularly wider bored and shallow) where either sunlight has penetrated into the well or the algae have adapted to compete in the dark with the "normal" microorganisms!

The concepts used in the ALGE-BART tester are based upon a crystallized dried nutrient pellet set into the base of the inner vial. Here, the nutrients gradually diffuse into the water. The chemicals are dominated by phosphorus with a minimal supply of nitrogen. This was done to encourage the activity of the algae that are able to fix nitrogen. These nitrogen fixing algae often dominate in surface waters as algal

TABLE B.5
Interpretation of Reaction Types for the ALGE-BART

Type	Color Type	Description	Major Algal Genera
GG	Grass green	Generally as a continuous lateral growth occurring at the water line	*Chlamydomonas*
FG	Fuzzy green	Patches of green growth at the water line but with fuzzy edges and sometimes indistinct	Many genera in the *Chlorophyceae*
OB	Orange to brown	Growth tends to be above the water line in the porous media as orange, red or brown patches	Commonly associated with diatoms and desmids
YB	Yellow to beige	Fuzzy patches of growth often above the water line may appear to be green-yellow initially but rapidly become yellow or beige	*Scenedesmus*
GF	Green flocculant	Much of the growth here occurs within the water as green flocs and/or deposits while the porous medium remains white	*Chlorella*
DG	Dark green	Growth begins a lateral green at the water level but the color rapidly goes darker and can even turn black. Dark green dense floc can also occur on the floor of the tester	Blue-green algae, *Cynaobacteriaceae*

blooms form and the natural nitrogen supply diminishes with the demands from the algal biomass. By creating nutrient diffusion gradients along the sample, the objective is to provide a series of niches where different algae are able to grow and flourish. To further encourage the activity and growth of sessile algae, the lower two-thirds of the inner tester in lined with a porous medium that presents a large surface area over which these algae can grow. Additionally, the porous (textile) medium also provides a "shadow" in the water sample that lowers the intensity of the light and encourages the growth of those algae not able tolerate high levels of illumination. The basic patterns of growth which can appear as a result of algal activity vary depending upon the types of algae that are present and active in the sample. The source of light for the growing of the algae in the sample may come from any one of these possible sources. These may include either A + B or A + C:

A. Northern natural light supplemented with either option B or C to assure 24 h a day illumination. If natural light is used without supplementation then there is a likelihood that the growth of the algae will become delayed and the TLs (days) to the observation of growth would be lengthened.
B. Tungsten light can be used preferably using a 40 W lamp placed at no closer than 60 cm and no greater than 100 cm away from the tester. Note that significant heat can be generated from tungsten lights and this could cause localized heating unless the area in which the test is being conducted is well ventilated.
C. Fluorescent light can be used but only if it is a natural or daylight type of light. For this testing the lights may be placed within the range from 150 to 200 cm above the testers with a wattage not exceeding 60 W, or at least 40 cm to the side and slightly above the tester.

Note that algae vary considerably in their ability to grow at different light intensities and wavelengths. This would mean that the conditions may be suitable for the growth of only some of the algae in the sample but not necessarily all of them. For example, sessile algae growing close to, or in, sediments and soils tends to have a lower tolerance for high light intensities and too much illumination may actually suppress their growth in the tester. To compensate for that then the distance to the lights should be placed at the greatest distance listed above for sessile algae while for plankton then the shortest distance can be used.

While the TL gives a prediction of the population of algae in the water sample being tested, the reactions observed that triggered the TL along with secondary reactions indicate which types of algae are dominant in the sample and are able to be active in the ALGE-BART tester.

B.10 PURPLE CAPPED ACID-PRODUCING BACTERIA—APB-BART

Acid producing bacteria (APB) are formed by a variety of heterotrophic bacteria that share the common ability to produce organic acidic products when growing under

reductive conditions utilizing organics. These APB cause the pH to drop significantly from neutral to acidic conditions ranging from terminal pH levels from 5.5 to as low as 3.5. These mildly acidic conditions are sufficiently corrosive to be significant to the integrity of any metallic structures being impacted. Because of these acid-producing activities in the absence of oxygen, it has been found that the APB are very likely to be significant partners in corrosion with the SRB particularly in the oil and gas industry. As a result, the management and control of corrosion frequently involves assessing the aggressivity of both the APB as well as the well-recognized SRB.

As a result of industrial practices over the last century it has always been considered that microbially influenced corrosion (MIC) were dominated by the SRB because of their ability to trigger electrolytic corrosion of metals. This event occurred primarily under highly reductive conditions in the presence of adequate sulfates and organics. In general, SRB generated hydrogen sulfide (H_2S) as a metabolic end product and it was this that triggered the corrosive processes. While this corrosion was primarily electrolytic, it was observed that some MIC was acidulytic and was caused by bacteria able to generate acidic products generally under highly organic and reductive conditions. It should be noted that little is known of the complex natural activities that occur in microbes with respect to the intelligent manipulation of electrolytic forces as a normal metabolic function.

Today it is recognized that the APB are significant contributors to corrosive processes through a gradual dissolution of the metal under the very acidic conditions that are created. In general these APB communities are found to be active under reductive conditions within biofilms, slimes, encrustations, nodules, and tubercles. Their activity can sometimes be noted as a lateral erosion of the metal surface that can be most clearly seen if the metal surface is examined using reflective light. Much of the APB is usually located at the metal–biomass interface. If present, the surface of the metal will appear to have an irregular pattern of shallow depressions. This would mean that the most effective examination of a sample for the presence of APB would be achieved by sampling the slime/concretion/encrustation/nodule/tubercle immediately at the interface between the growth and the metal surface. This is different to the electrolytic corrosion caused by SRB which tends to cause deeper pitting of the metals and deeper cavities. In simple terms, the APB generally cause broad impacts over much of the metal surface while the SRB cause focused forms of pitting and cavitation within the metal.

Acting producing bacteria APB always generates organic acids that commonly do not drop the pH below 2.0 but there are other bacteria that generate inorganic acids (commonly sulfuric) as a result of the oxidation of sulfides and sulfur. These bacteria are associated with acid mine drainage and cause the downstream waters to become very acidic primarily because of the oxidation of sulfides and/or sulfur to sulfuric acid. The major genus associated with these activities is *Thiobacillus* and is commonly known as the sulfide oxidizing bacteria (SOB) and they can be detected using the SOB-BART (if the oxidation is primarily driven by sulfides) or SUOB-BART (if oxidation is primarily driven by sulfur). These bacteria are commonly associated with not just acid mine drainage from sulfide-rich tips and tailings but also sulfide-rich soils where the problems occur because of the very acidic

conditions that can corrode metals and concretes. In Australia, the acid sulfur soils are causing billions of dollars of damage each year. Water wells containing highly active populations of SOB or SUOB communities are likely to have very low (acidic) pH values in the water. Acid producing bacteria can be active within a well but the water may still have a neutral or slightly alkaline pH since these bacteria are active within the biomass and not the water.

To conduct the test, the APB-BART tester is placed upright at room temperature and observed on a daily basis. If APB are present in the sample and become active then there will be a drop in the pH in the lower part of the tester (the more reductive region) and the color of the culturing fluid will shift from purple to a dirty yellow (DY). Determination of a positive for APB is recognized when a yellow front begins to rise up from the floor of the tester. This should not be confused with the time early in the test when the crystallized medium is dissolving and there may be a small zone of dark yellow forming in the base of the tester. This color change to DY indicates that the pH has dropped to less than 3.5–3.9 and it will progress over time until the bulk of the liquid in the tester has turned yellow. A TL can be declared when there is a clear sign that the yellow (acid) is moving up the tester from the base 5 mm up the side wall above the base cone of the tester and can be used as the indicator that a DY reaction is occurring.

B.11 YELLOW CAPPED FLUORESCENT PSEUDOMONAD BACTERIA—FLOR-BART

The FLOR-BART tester has been specifically developed to detect heterotrophic bacteria belonging to species of the genus *Pseudomonas*. Some species have been linked to increased health risk while others dominate bioremediation processes through their ability to degrade organic pollutants efficiently under oxidative (aerobic) conditions. When water samples are tested using the FLOR-BART, the main focus has to be on the production of colors that fluoresce in UV light. There are two major colors that are detected:

PB—Glow in UV Light.
GY—Glow in UV Light.

These two reactions relate to particular species of *Pseudomonas*. For the PB reaction this will be seen mostly around the BART ball extending generally 2–6 mm beneath the ball. This color reaction occurs between 2 and 4 days after the start of the test and then fades. When at its brightest, the PB can be seen in regular room light. For the GY reaction this will be seen mostly around the ball extending generally 4–10 mm beneath the ball. This color reaction occurs between 3 and 10 days after the start of the test and can last for as long as 2 weeks before fading.

If there is a PB reaction then that means *P. aeruginosa* likely to be present. These species present a potential health risk since *P. aeruginosa* can cause infections in humans. These infections can range from pneumonia to eye and ear infections through to skin and lung infections. It is therefore recommended that, when PB is

observed, confirmatory test be performed using a certified microbiology analytical laboratory. If a GY reaction is observed then species belong to the *P. fluorescens* group are likely to be present. These species frequently dominate waters where there is a significant amount of aerobic degradation of specific organics taking place.

B.12 COMPARISON OF BART TO OTHER TEST METHODS

BART testers provide a relatively simple method to detect specific microbes and at the same time get an appreciation of how active that population is. This section of the chapter deals with the comparisons that can be made between the BART testers and other (mostly laboratory-based) microbiological test methods. Inverse correlations have been found between the TL and population size that makes the BART tester system superior for monitoring work using the TL as the primary indicator of the level of microbial activity occurring in the sample.

B.12.1 Comparisons of BART Testers and Heterotrophic Plate Count and Adenosine Triphosphate Tests

There has been some suggestion that the BART technologies cannot be compared with the ATP (Adenosine Triphosphate) and the HPC (Heterotrophic Plate Count) methods. Table B.6 presents a direct comparison of the HAB-BART tester with the ATP and the HPC methodologies showing the relative advantages and disadvantages of the three methods.

All living cells produce ATP as the main mechanism to store energy in the cell. When a sample contains many active microbial cells then there is much more ATP present. Testing for ATP in a sample has become the "gold standard" by which the numbers of active cells in the sample can be counted. BART testers use a similar approach but here the activity is measured by the TL to a recognized reaction or activity rather than the concentration of ATP. Here a shorter TL would mean more activity and higher ATP levels. Studies at the Universities of Western Ontario and Saskatchewan have found good semi-quantitative correlations between the concentrations of ATP in water and soil samples and the TLs generated by BART testers. BART testers are, however, simpler to set up than the ATP analysis, less expensive, and can also be used away from a laboratory setting. Time lapses taken from the BART tester can provide good correlations to the ATP analysis but are more economical and more convenient in their use while at the same time obtaining good precision (Table B.7).

B.12.2 Environmental Technology Verification (Canada, ETV)

BART technologies have been subjected to evaluation in Canada and three systems have received Environmental Technology Verification (HAB-, SRB-, and IRB- BART methods). They have been in use in a routine manner since

TABLE B.6
Comparison of HAB-BART System to Heterotrophic Plate Count (HPC) and Adenosine Triphosphate (ATP)

Feature	HAB-BART	ATP	HPC
Target	All active heterotrophic bacteria in sample	All active prokaryotic or all cell types active in sample	All active heterotrophic bacteria able to grow on agar
Initial Introduction	1991 with Canadian ETV in 2003	1980s with EPA acceptance	1930 with modifications through to 1980s
Differentiates aerobes from anaerobes	YES	NO	NO
Field applicability	YES can be used at the field site	YES if some of simpler analytical systems are applied	NO, has to be set up in a certified laboratory setting
Time length for testing	Up to 5 days using the HAB-BART system	Rapid once in the methodologies have correct settings	1–4 days, requires skilled operators
Operator skill level	1 day training	Certified technologist	Certified microbiological operator
Sample handling	Test performed within 4 h at site	Sample shipped to certified laboratory	Sample has to be shipped to certified laboratory

1991 and have been adopted by the U.S. Army Corp of Engineers for the evaluation of the effectiveness of treatments applied to all kinds of water wells. Further information is available at the web site www.dbi.ca. It should also be noted that:

1. Further information on the BART systems can be accessed from *Microbiology of Well Biofouling* by Roy Cullimore published by Lewis Publishers/CRC Press in 1999 and also *Simplified Atlas for the Identification of Bacteria* by Roy Cullimore published by Lewis Publishers/CRC Press in 2000.
2. Comparison of the BART systems with the various other microbiological test methods is listed on the web site www.dbi.ca.
3. Over the history of the use of BARTs they have been proven to be reliable and sensitive to active bacterial populations. This feature has caused some problems where companies are making overly optimistic claims for the treatment products then the BART system shows a null (zero) effect. Then it is a case of "shoot the messenger" rather than address the treatment protocol. It should also be noted that it takes 4–8 weeks for a well that has been effectively treated to again stabilize and show consistent results. Testing samples immediately after treatment

TABLE B.7
Semi-Quantitative Projection of Population Size (p.a.c./mL) Based on Daily Determinations of Time Lapse (TL) for the SRB-, SLYM-, IRB-, and HAB- BART Testers

		TL (days)				
		1	**2**	**3**	**4**	**8**
SRB-BART	Maximum	50,000,000	15,800,000	539,000	36,600	68
	Mean	23,200,000	731,000	46,600	5,200	31
	Minimum	999,000	59,700	6,340	1,100	16
		TL (days)				
		1	**2**	**3**	**4**	**5**
SLYM-BART	Maximum	50,000,000	6,620,000	468,000	57,400	9,440
	Mean	8,800,000	632,000	70,000	11,100	2,400
	Minimum	805,000	85,700	13,200	2,760	749
		TL (days)				
		1	**2**	**3**	**4**	**6**
IRB-BART	Maximum	1,000,000	492,000	123,000	30,700	191,000
	Mean	566,000	141,000	36,300	8,800	850
	Minimum	162,000	40,500	10,100	2,530	158
		TL (days)				
		1	**2**	**3**	**4**	**6**
HAB-BART	High	40,000,000	5,200,000	355,000	39,100	6,290
	Mean	6,900,000	454,000	47,800	7,400	1,590
	Low	563,000	59,000	8,830	1,800	502

Note: TLs are listed from days 1 to 8 as the maximum with the potential population range is shown based predicted active cells/mL with the semi-quantitative accuracy with the high value set at 1 h after the previous reading and the low as a positive just before the reading on the next day. Population predictions are based upon BART–SOFT version 5 with room temperature incubation.

can show vacillations from a total kill to a stimulation of active bacterial numbers. In any regulated monitoring of a treatment this is a very important consideration (Table B.8)

B.12.3 Comparison of BART Testers with Other Microbiological Tests

BART testers can use the water sample directly or they can accommodate solid or semisolid samples. No dilution is employed if it is a liquid sample with a low turbidity.

TABLE B.8
Semi-Quantitative Projection of Population Size (p.a.c./mL) Based on Daily Determinations of TL for the DN-, APB-, BRB-, and POOL- BART Testers

		TL (days)				
		2	**3**	**4**	**5**	**6**
DN-BART	Maximum	5,000,000	180,000	13,600	1,790	362
	Mean	242,000	17,200	2,140	417	115
	Minimum	21,800	2,580	183	129	45

		TL (days)				
		1	**2**	**3**	**4**	**6**
APB-BART	Maximum	5,000,000	623,000	54,500	7,440	396
	Mean	817,000	68,000	8,920	1,690	143
	Minimum	85,400	10,700	1,970	493	63

		TL (days)				
		1	**2**	**3**	**4**	**5**
BRB-BART	Maximum	20,000,000	13,200,000	1,660,000	271,000	55,600
	Mean	16,500,000	2,020,000	321,000	64,500	15,800
	Minimum	2,460,000	382,000	75,000	18,100	5,230

		TL (days)				
		1	**2**	**3**	**4**	**5**
POOL-BART	Maximum	10,000,000	3,450,000	196,000	19,300	2,950
	Mean	4,760,000	255,000	23,800	3,500	740
	Minimum	332,000	24,500	4,160	852	235

Note: TLs are listed from days 1 to 6 as a maximum with the potential population range is shown based predicted active cells/mL with the semi-quantitative accuracy with the high value set at 1 h after the previous reading and the low as a positive just before the reading on the next day. Population predictions are based upon QuickPop version 3.1.

The tester is commonly incubated at room temperature although 28°C is faster and generates a greater precision. Where a liquid sample is used, the tester accommodates 15 mL for the test and the examination is for any activity or reactions within that tester caused by any of the microorganisms able to grow in the sample. For the solids or semisolid samples (such as soils and muds), 0.1–0.5 g are used diluted into a sterile diluant. In these tests where the sample is not subjected to any dilution (i.e., not solid or semisolid) this minimizes the impact on the microorganisms in the sample and improves a better potential for effective recovery. Within the BART tester a variety of environments are established to increase the

potential for activity and reactions to be observed. Comparisons to the BART testers described below include:

Agar Spread Plates. Use agar to make a jelly-like base upon which the bacteria can grow to form colonies (visible growing piles of cells) that are often easy to count and, with the right agar, can also be used to identify the types of bacteria that are present. That is why the counts using agar refer to "colony forming units" also known as "c.f.u." The problem with the agar plates is that the microorganisms have to grow on the agar into a recognizable form as a distinctive colony. While this method has a lot of convenient features, it does not detect the many types of bacteria that are not able to grow on agar surfaces, that cannot tolerate the high levels of oxygen, or cannot extract water effectively from the agar. BART testers have the advantage in that many more types of microorganism can grow in the BART tester often much faster because of the greater variety of environments that the tester presents to the organisms in the sample. BART testers are therefore more sensitive to a wider variety of microorganisms than agar-based media and can generate shorter delays before growth occurs.

Agar Dip Paddles. These are constructed using a relatively thin agar film over a plastic paddle that is immersed in the water sample to inoculate the agar surface with microorganisms from the sample. Challenges for this technique are that the microorganisms have to become attached to the agar surface and then subsequently grow to form visible colonies when the dip paddle is incubated. Problems with this technique are that the agar forms a fairly thin film over the plastic and can begin to dry out quickly thus increasing the concentration of chemicals in the agar and reducing the range of microorganisms that would effectively be able to form and grow colonies. BART testers, in using 15 mL of sample, have a greater potential to cultivate such bacteria efficiently and therefore produce more precise data without having to be concerned about the agar drying out and giving inaccurate results.

Quick Tests (*Color Change*). There are a range of fast microbiological tests that claim to detect the numbers of bacteria in a semi-quantitative manner (e.g., a lot, a few, and none). Such tests usually involve filtering or contacting the sample in order to trap the microbial cells that are then stained by some colored chemical reagent that reacts to these cells. The more the cells present on the filter, the more intense is the color. The problem with these techniques is that filtration means concentrating all of the organics (live and dead) onto the filter. The reagent may become reactive to the dead organic matter and signal a falsely high color reaction beyond the actual number of active cells present. While these tests are fast, they lack the precision of the BART tester. Some BART testers such as the HAB-BART tester can react within a matter of 3000–5000 s when there are very large active populations of bacteria present (such as in primary influent from a sanitary waste water treatment plant) and can be

TABLE B.9
Semi-Quantitative Projection of Population Size (p.a.c./mL) Based on Daily Determinations of TL for the ALGE- and FLOR-BART Testers

		TL (days)				
		1	**3**	**6**	**12**	**18**
ALGE-BART	Maximum	10,000,000	829,000	74,700	2,010	173
	Mean	2,270,000	383,000	39,500	1,300	129
	Minimum	991,000	185,000	31,700	870	98

		TL (days)				
		1	**2**	**3**	**4**	**5**
FLOR-BART	Maximum	10,000,000	3,450,000	196,000	19,300	295
	Mean	4,760,000	255,000	23,800	3,500	740
	Minimum	332,000	29,500	4,160	852	235

Note: TLs are listed from days 1 to 18 as a maximum with the potential population range is shown based predicted active cells/mL with the semi-quantitative accuracy with the high value set at 1 h after the previous reading and the low as a positive just before the reading on the next day. Population predictions are based upon QuickPop version 3.1.

reproducible. BART testers are quick tests too when there is a very large and active bacterial population in the sample.

ORP. A comparison to the reactions and activities of the detected microorganisms in the samples does show links between the reaction and the ORP in the sample (Table B.9).

Immunoassay tests, *comparison to the BART testers*. A whole new science called immunology was developed when it was realized that microorganisms all developed unique chemical signals. Some of those signals were specific to disease producing microorganisms and caused a specific response in the body enabling the body to become resistant (immune) to the pathogen. Today a whole biochemical science has evolved so that many microorganisms can be very precisely identified by unique chemical signals carried by the cells of a specific species. Some of these chemicals are called antigens and the infected body produces antibodies that neutralize them. By applying these antibodies wearing color-coded tags, it is possible to detect specific species of bacteria. These tests are more effective in a laboratory setting with highly trained technologists, however, some field tests do exist although there are often many precise steps in conducting the test. For example, an immunoassay test for SRB can involve six or more steps, precise timing, and various apparatuses to achieve a result. The highly precise technique used in the field test opens the door for a variety of errors to occur, resulting in false positives, or negatives. BART testers examine samples for whole communities of

bacteria looking primarily for the quantification of the activity levels (population size) and reactions that are achieved (qualitative determination of the types of bacteria present). Immunoassay tests are designed to detect specific species of bacteria in the sample. In most samples there are active communities present that can include as many as 8–16 species just in each of those communities and for these conditions the BART testers offer more information with a simpler test method. Immunoassay tests are generally both complicated and expensive while the BART testers are simple, economical, and can detect the nuisance bacteria causing the problems.

C Video Borehole Log

Color borehole television camera surveys, using either videotapes or digital recording, form an essential component of the historical information base for recording changes occurring in the condition of a well and as a diagnostic tool in the determination of the efficiency of well regeneration. These surveys should be taken at critical times such as immediately after well construction and at intrusive servicing intervals such as during preventive maintenance (prior to and following the treatment to determine effectiveness). These records may be consolidated showing important well features over the years, but each should be labeled by well identification and dated, then stored properly in an accessible location.

It is recommended that any video log survey should follow a standard protocol that allows an easy comparison of the imagery. This should include ensuring that the date, time, and position are always displayed together with the camera lens position in relation to the well screens. This latter function is particularly important in horizontal wells where the fouling events may be occurring laterally. For example, gas pockets would tend to collect on the upper arc of the screen where the media beyond the screen is fouled, and microbial encrustations may be localized over an arc of the screen that is consistent. This data also enables the user to locate particular regions in the well and determine whether a preventive maintenance or regeneration treatment has been effective at reducing or eliminating these growths or clogs within the body of the well. It should be remembered that the presence of a pristine screen and borehole does not mean that the well is fouled but simply that there is no visible evidence of fouling within the well. There would only be visible evidence where the fouling has become intrusive in the well borehole. Historical records denoting changes in the specific capacity (Q/s), camera logs, and water quality parameters (including relevant biological parameters) can be used to assess the status of any fouling occurring within the injection and extraction wells. There would be differences in the manner in which the data is obtained depending upon the nature of the individual well. Extraction wells would be easier than injection wells to determine the Q/s since the normal hydraulic flow would be into the well during operations. For injection wells, the Q/s should be obtained using the flow into the well.

Often considerable fouling may occur within the well itself and the rate at which is occurs can be determined by routine (e.g., annual) video camera logging of the well. If the logging is standardized then a review of the images at specific depths and/or locations down the well can provide evidence of the rate of visible fouling. In addition the effect of regeneration and other treatments on the rate of fouling of the

system can be determined. These camera logs of the well can over time give significant amounts of information about the problems that are generating within the well. For example if the well has been subjected to a physical failure such as a screen separation from the casing then this can be easily recognized in the video log. Additionally, comparisons of these video logs can sometimes clearly indicate when a well is failing with fouling.

In comparing historical video-camera logs of the same well it is essential to ensure that common measurement frames have been consistently used so that the position of the site of concern in the well can be determined for each of the logs covering this particular section of the well. It is considered essential that the camera records direct view of the screen and filter pack and it is preferred that the camera is equipped with an articulating 360° panning lens. It is equally important to ensure that the camera travels slowly in a consistent manner each time the inspection is conducted. A fast rate of descent with the camera speeds up the survey (making it more economical) but it can lead to difficulty in locating particular sites within the well and can also dislodge some of the fouling material into the water. Where this happens and the material breaks up, visibility is obscured reducing the value of the video records. The speed of video-camera movement can be varied at the initiative of the operator particularly if there is no evidence of any fouling (i.e., a clean borehole). In the event that there is evidence of fouling, the speed of the video camera should be slowed down to assure good clear images for the logged location. If cloudiness occurs in the water as a result of the knockdown and dispersion of fouling materials, the camera's movement should be stopped until the cloudiness has cleared. If the clouding will not clear then the camera should be slowly moved out of the zone. If the camera is being operated at per hour costs, there is always the temptation to save money by speeding up the movement of the camera. Where this is done then the savings do not match the loss of understanding of the state of the fouling and getting a good understanding of the health of the well.

Golden rules when using the camera are:

1. Go slowly where there is significant fouling in the well otherwise the disruption could lose the viewing capacity.
2. Remember that most of the biological activity is likely to be beyond view and so look carefully laterally into any slots, fractures, and perforations and look for closed voids and growths.
3. Water itself is an indicator of the health of the well since older wells will tend to show more particles hanging in colloidal (jelly-like) water and these are likely to be layered, therefore the following.
4. When descending into the well with the lens going straight ahead (downwards) then it is possible to see these jelly-like states where the particles are just hanging in the water.
5. When getting towards the bottom of the well, remember that banging into the sediment collected on the floor could lead to a prolonged loss in visibility, therefore draw up slowly from the base of the well.

If video-camera logging is to be conducted of a horizontal well then different concerns exist. When video camera logs the horizontal wells, as opposed to vertical injection and extraction wells, then there will be some configuration challenges. In the horizontal wells, gravity plays a major role in the positioning of biofouling materials and products. Additionally, horizontal wells may pass slowly through lateral redox fronts that can affect a considerable length of the screen. Gases, for example, will tend to collect under the upper side of the borehole where the slots and the porous media beyond have become plugged or clogged. This entrapped gas would be seen as a ribbon of gas rippling along the upper side of the well. Additionally, growths may be seen hanging down rather like stalactites into the water while others may from complex growths involving calcite and/or iron oxides and hydroxides (goethites). A careful note should be made of the positions of these growths and the occurrence of perched gas pockets so that after regeneration or preventative maintenance, any regrowth of this biofouling can be recognized and positioned. An effective treatment should prevent regrowth at the same locations and the plugging events should again be random.

Vertical wells tend to have stratified events at different depths down the well relating to the oxidation–reduction potential and the entry of various materials down the length of the vertical column. For example, in a contaminated well, a floating plume would appear focused on the surface of the water column and may have elements of biofouling growing above (on the walls of the casing), within the plume and/or below the floating plume in the water. If the floating plume is able to allow oxygen diffusion (e.g., diesel fuel) then this growth would be underneath the plume and can become intense and obscure any images of the well's walls at those depths. Further down in the well's water column, evidence of growth may appear to be cloudiness, slimes, encrustations, nodules, tubercles, and even crystalline calcite-like structures occurring at specific depths. These depths usually relate to the points where groundwater is now, or has now been plugged off by the growths, entering the wells through the screen slots or directly from the fractured or porous media. In some cases these slime-like growths can cross connect at a specific depth looking like a false well bottom. Often lightly tapping this "slime" floor with the camera can cause it to collapse. The position of this false floor can reflect the position of the redox front in the well. Where this is the case, above the floor will be oxidative while beneath the floor will be reductive. On the reductive side there is likely to be an accumulation of gases from biological activity including, but not specifically limited to, methane, carbon dioxide, and nitrogen. When the "slime" floor is penetrated (for example by the camera), this entrapped gas may suddenly be released (erupt). The gas will then move up the well as a series of large bubbles easily seen using the video camera. Moving the camera to below this "slime" floor into the reductive zone underneath will now reflect the reductive nature of the environment. The walls will tend to be blackened with sulfide and iron carbonate deposits and the growths (where observed) would have a gray or black color as either soft slimes or hardening encrustations. There is less stratification at these levels and the water is often less turbid (clearer).

Video-camera logging of the horizontal or vertical injection or extraction well gives a considerable amount of information about the structural integrity of the well

and also the form of biofouling that may be occurring. By historically examining the various camera logs it then becomes possible to get an appreciation of the sequences of fouling in the well appeared, and also the likelihood of these materials being of a biological origin. If the materials are slime-like then there is a virtual certainty that they are microbial. Where the materials appear hardened but break up upon contact releasing clouds of colloidal (slime-like) particles, these are also microbial. Even hardened encrustations, if they have softer zones within, are likely to be microbial. Very hard crusts and crystalline materials would, at first examination, appear to be geochemical in origin, but on some occasions these are actually the products of microbial activity.

D Treatment Strategies

D.1 INTRODUCTION

Perhaps one of the prime factors in the decision to treat a well, once the treatment alternatives have been compared and a selection made, is whether the treatment is going to be done in-house or by an experienced contractor to apply the various technologies as required to treat the well. Great care should be taken during the selection process to have contract language that requires a minimum of 5 years experience in applying well regeneration technologies and 1 year applying the selected treatment method. Inexperienced contractors can often do more harm than good to wells! It is therefore important that whoever is the contracting officer for the treatment should check references from previous customers of each contractor. While cost is certainly an important factor in selecting a treatment option, it should not be the overriding factor.

There are many inexpensive ways to rehabilitate wells that are, by and large, unfortunately ineffective. It has to be remembered that the biomass that has formed in, and around, the well is normally set into fractures and porous materials that are difficult to penetrate chemically. This is particularly the case for finer grained materials such as sands that still have a relatively high porosity of 25%–35%. Treatments designed to penetrate into these formations have the major challenge that the chemicals have to penetrate through the narrow throats into small voids that are coated, if not packed, with biomass. By comparison the borehole itself is relatively vulnerable to both physical and chemical treatments. This means that a treatment may appear to have totally cleaned the borehole with the casing and screens looking pristine. That does not mean to say the problems are solved particularly if the major part of the plugging biomass is set back in the formation away from the borehole. In these events, the camera video logging after a treatment may show an efficient cleaning of visible surfaces. This does not mean that the well has been effectively treated particularly if the Q/s has not improved. Another common problem that comes from treating the water well stems from the fact that the biomass perched around the well is not just accumulating iron and other metallic cations and potentially useful organics but is also trapping sands, clays, and silts within the biomass. These relatively inert materials simply collect within the biomass and can sometimes gradually become bioconcretions. Such inert accumulated materials add an extra problem to the treatment of the well since these inert materials significantly affect the ability of the treatment to penetrate deeply into the biomass. Furthermore, when a treatment has been successful then

these inert materials may be released out of the biomass to cause problems downstream, particularly when settling into the borehole. One of the problems of effectively destroying the biomass around the well is to ensure that these released sands, clays, and silts do not enter and settle in the well.

For the operators of wells affected by these perched inert materials entering the well, the success seen in the treatment is sometimes followed by disappointment when the borehole fills commonly with sand for a significant distance up the borehole. In the treatment of the well, it is important to include physical force through surging the well to remove this sand. When treating wells that are suffering from a perching of particularly sands from within the plugging biomass, there is a need to be prepared to use physical agitation in order to bring up the sand and fines and remove them from the well. These releases may continue for some time even after the treatment itself has been completed and there must be a contingency plan to remove this material as it enters the well. The fact that material enters the borehole is actually a good sign since it means that these sands and fines are no longer locked in the biomass in the formation and, furthermore, the treatment is working. Choosing the right treatment is not an easy task and the risks of success have to be considered along with the advantages of improved Q/s and reduced biological activity in the well.

D.2 TREATMENT ALTERNATIVES

Perhaps the first thing to remember is that no two wells will foul in exactly the same manner. To have a successful regeneration strategy then the contractor must take into account the nature, location, and form of the fouling that is occurring in each individual well. This would mean that it is not appropriate to assume that all of the wells in a given field should be treated exactly the same way. Because each well will have a different sphere of influence, be set into slightly different formation material, and be constructed separately to the other wells, there will be variations that should be taken into account prior to, and during, the treatment. This may be particularly relevant if the wells are to be subjected to regeneration.

The industry sector involved in well regeneration has a natural bias towards the application of their recommended treatment products and processes. Regeneration, unlike replacement, of all types of water wells remains a relatively young service industry and this has resulted in the limited number of viable options available and a limited database of independent verifications of the effectiveness of particular regeneration technologies. One initial strategy that can work well is to physically remove the attached biomass growing inside the well by jetting or brushing. All of the biomass removed at this early stage reduces the challenges and costs of applying more rigorous treatments to do the same thing (i.e., remove the internal growths) from the well. Appendix D will be divided into two parts:

1. A survey of the potential treatment applications that are currently in use.
2. A discussion as to which of the potential technologies would be the most suitable for the particular problems in the extraction, injection and horizontal wells of direct concern.

This includes both the critical steps in diagnosing the problem, the selection and application of the treatment, and the formulation of post-treatment preventative maintenance servicing.

D.3 SURVEY OF POTENTIAL TREATMENT APPLICATIONS

Regeneration treatments can often involve some combination of physical and/or chemical applications since the target is to control, and preferably remove a form of fouling that is dynamic and involves different agencies. At this time, regeneration is focused on physical and chemical treatments because no biological treatment has yet been demonstrated to be effective in the long term. Two classical approaches to biological treatment are the use of antibiotics, or the application of "bugs in the box." Neither of these methods has proved to be effective. For the antibiotic treatments, the biomass is very large and diverse which limits the ability of the antibiotic to be effective. For the application of bacteria to a biofouled well, the challenges to the successful infestation and destruction of the plugging biomass are enormous. It would mean that the tailored bacteria being applied to the well would have to compete and win against a minimum of at least three different microbial communities.

In this survey of treatment options, there are three major sections covering the applications ranging from the use of a single physical or chemical treatment to the blended treatments that involve both physical and chemical aspects. Selection of a treatment option to achieve the successful regeneration of a well will be somewhat different when the problems originate from geochemical clogging sources rather than biological plugging. The treatments' relative effectiveness will be addressed for each of the potential fouling conditions.

D.4 OVERVIEW OF PHYSICAL TREATMENTS

Physical treatments can be divided into the following categories:

1. Direct application of heat.
2. Hydraulic surging and direct physical cleaning of the fouled sites which could involve some form of sonic pulsing.
3. Shifting in position of the redox front.
4. Application of electromagnetic charges.

These are addressed separately below.

D.4.1 Direct Application of Heat

Since the discovery of the use of heat to control the biofouling (spoilage) of foodstuffs in the earlier part of the nineteenth century by sterilization (Nicholas Appert) and later pasteurization (Louis Pasteur), heat has been widely used

particularly in the medical, food and beverage industries to control biofouling/spoilage. All living organisms can only function within a set range of temperatures. Elevating the temperatures outside the "comfortable" growth range inhibits organisms and becomes lethal once the temperature rises well above the normal growth range. In regeneration of biofouling, it was considered that simply raising the temperature by greater than 40°C (104°F) above the ambient background temperatures was normally sufficient to kill off the cells that do not have heat-resistant forms (e.g., spore). The advantage of applying heat to a well is that a thermal gradient will form and gradually move out around the well, penetrate the slimes and deposits as well as the porous media and eventually kill most of the microbes contained in that zone. This technique can be a very effective method to reduce the microbial population.

Various techniques have been applied to heat wells as a means of controlling plugging including injected steam and hot water, the use of immersion heaters, and the use of chemicals that generate exothermic reactions when hydrated. A relatively small number of biofouled wells were found to be recoverable using this technique even when the temperatures reached as high as 85°C (185°F). The reason for the relatively poor performances observed was considered to be a combination of failure to disrupt and disperse the biomass and other deposits along with the thermal coagulation of the slime (similar to the impact of boiling the white of an egg). The application of heat has been found to be more effective when combined with chemical treatments since this additional heat also accelerates the rate of the chemical reaction causing shock and disruption of the fouling material within the well.

While raising the temperature in the fouled zones in, and around, a well has been investigated, there have also been some efforts to lower temperatures in order the freeze the biomass. It has been documented that when a biofilm freezes, it will commonly detach and become disrupted. Because of the technical difficulties of freezing a fouled zone around a well, it has never been developed to a fully commercially verified technology. Similar to the blending of heat with chemicals to improve the effectiveness of regeneration has been effective and so blending low temperatures with chemical additives (such as carbon dioxide) can, under some conditions, prove to be effective for regeneration.

D.4.2 Hydraulic Surging and Direct Physical Cleaning of Fouled Sites

Geochemical clogging often involves siltation with fines (silts, sands, and clays) within the voids in response to the water flow patterns. When this is the principle problem, radically changing the water movement characteristics, usually by vigorous agitation within the borehole, can set up conditions that disrupt and disperse this material. Agitation can either push the material back out further into the formation or release the material into the borehole where it can be removed by techniques such as air lifting. Biological plugging, when also significantly present, tends to reduce the ability of physical surging to release the accumulated materials

due to bioconcretion of the fines. This is principally due to the binding action of the polymeric slimes. In the event that the problems in the wells involves mainly a biological form of plugging then the hydraulic surging would be more effective after the plugging material has been shocked and at least partially dispersed by some physical–chemical method such as through the use of heat and effective penetrants. Once the material forming the biological plugging has been dispersed then surging can much more effectively remove the bulk of the biofouling within the treated zone.

In addition to surging, the direct action of brushing down the screens in a borehole is recommended where there is material in the form of encrustations, slimes, or deposits that is adhering to the insides of the screen slots. Brushing utilizes a simple series of whip-like strokes to physically detach such materials that may then be removed by hydraulic or air-lift surging of the well. Essentially the initial brushing will remove some of the more exposed materials from the well and open up fresh surfaces for the other treatments when they are applied. There are advantages in brushing down the screens before any other treatment since this removes some of the biomass and concreted fines without wasting the more expensive treatment chemicals on doing the same job.

D.4.3 Shifting in Position of Redox Front

In laboratory experiments on the deliberate biofouling of porous media, it is common for the biofilm growths to concentrate around the redox front. As discussed previously the redox front forms a natural focus for microbial activity because it is at the interface between oxidative (oxygen-rich) and reductive (food-rich) conditions. Where the redox front is close to the well (as may be the case with many horizontal and injection wells) then there would be a significant increase in biomass close to the well. With the growth and maturation of this biological plugging, the specific capacity can become drastically reduced. It has been found that where the redox front sets up further back from the borehole, there is a larger amount of available surface area and void volumes within which the biofouling can grow. As a result there is a much slower impact on the specific capacity of the well.

Some techniques deliberately involve, as a regeneration treatment, the relocation of the redox front further away from the well. This can be done by creating a larger oxidative zone in, and/or, around the borehole. A larger zone can be obtained by injecting aerated water down a number of satellite wells around the affected well, or by injecting the aerated water directly down the borehole often at rates equivalent to a significant part (e.g., one-third) of the production of water from the well. In either case, it is the expansion that occurs in the size of the oxidation zone around the well that drives the growth of fouling microbes further out into the formation. Forcing the redox front out could be compared to blowing up the "slime balloon" so that it is further away from the well and would take longer to have an effect. Driving the redox front further back into the formation also increases the surface area of the front that would have to be severely fouled in order to affect the production characteristics of the well. The net effect is that the biological plugging moves outwards away from the well along with the redox front. This causes the

plugging to become dispersed over the greater surface area and the specific capacity of the well is not as dramatically affected by the ongoing plugging of the well. It should be noted that clogging because of its geochemical nature would not be so affected unless it is also driven predominantly by chemical oxidation phenomena. Some methods are the subject of patent protection.

This technique of manipulating the redox front is more applicable to improve the sustainability of the water well by reducing the rate of observable biofouling rather than to be employed as a regeneration technique on a situation where the biological plugging is already deeply entrenched around the well. It should be noted that the acquisition of a larger oxidation zone through the inputs of oxygenated water into the well environment could enhance the overall level of aerobic microbial activity. Where this happens the discharged waters may carry a greater burden of more aggressive bacteria that have sloughed from the larger surface areas generated by the expanded redox front.

D.4.4 Application of Electromagnetically Charged Surfaces

Biofouling and the associated microbially initiated corrosion have been a major concern in all industries using steel structures and pipes. In the oil and gas sector these problems can be catastrophic leading to major losses of product and many installations employ an active cathodic protection to the vulnerable surfaces. Corrosion, where it is microbially induced, involves a number of stages. The early stages relate to the attachment of microbes to the surfaces and the generation of expanding biofilms that cover significant amounts of the surface. The forms that the biofilms mature into are very much a factor of the local environment including the form of any metals and concrete at the surface and their physical state (e.g., porous, embrittled, fractured). Growths may generate into nodules, tubercles, and encrustations and may be either iron- or calcite-rich. Corrosion is mostly initiated through the biofilm-generating acids (acid-producing bacteria) or hydrogen sulfide (sulfate-reducing bacteria). When the latter is produced, the gas initiates an electrolytic form of corrosion. Corrosion can cause a number of effects from pitting, lateral fracturing, dramatic losses in strength, and mechanical failure.

To understand corrosion there is one golden rule that microbes are all negatively charged and therefore will normally gravitate towards positive charges. When cathodic protection is used to protect a surface from a microbial infestation, the surface contains an impressed negative charge that prevents successful attachment of microbial cells to the surfaces. At the same time an anodic (positively charged) surface is generated as a sacrificial point at which microbial attachment and subsequent growth and corrosion will occur at sites that have no consequence to the operation of the device. The art of cathodic protection is now widely practiced but has not been extended to the injection and extraction wells because of lack of experience, high operating costs, and uncertain benefits.

This use of cathodic and anodic charged surfaces and fields is now being subjected to investigation as a means of anodically focusing or cathodically disrupting (CD) a potential or actual site of biofouling. Some aspects of these processes are now being subject to intellectual protection and have potentially major

implications for application to wells as a means of manipulating the form, function, and position of biofouling events. It is expected that the CD process may be very suitable for application as a part, or the whole, of a preventative servicing treatment.

D.5 OVERVIEW OF CHEMICAL TREATMENTS

There exists a broad spectrum of chemical treatments, some of which are target specific single chemical treatments while others involve applications using various blends of chemicals. For the purposes of this survey, the chemicals are separated into the following sections:

1. Disinfectants.
2. Acids.
3. Dispersants/penetrants, alkalis, and chemical blends.

In all cases of chemical treatment there is the challenge of how best to dispense the chemicals.

D.5.1 Disinfectants

Disinfection relates to the ability of a chemical to significantly reduce or eliminate the risk of microbial infection arising from contaminated water. Chemicals are traditionally used to reduce health risks by suppressing or killing infectious microbial agents causing such diseases as cholera, dysentery, and typhoid. The perception that disinfectants are equally effective against all microorganisms is not necessarily the case. Traditionally, the effectiveness of disinfectants has been judged by their effectiveness against the enteric bacteria that includes the coliforms such as *Escherichia coli*. However, the focus in treating an extraction, injection, or horizontal wells should be on disinfecting the well of the infestation of microbes causing problems to the "health" of the well. Unfortunately, the disinfectants effective against the enteric bacteria may not be so effective against these biofouling microbial agents that may be very diverse in type.

Chemical groups commonly used as disinfectants include the halogens, various benzene/phenol-based compounds, and ozone or peroxides. Of the halogens, it is the chlorine-based compounds that have been the most effective particularly at controlling the enteric bacteria including coliforms although both iodine and bromine compounds have been found to be effective under some circumstances. There is a vast array of benzene/phenol-based compounds available in the marketplace and many function to suppress microbial activity (bacteriostatic) rather than kill (bactericidal) in the low parts per million range. Ozone or peroxides are both commonly used disinfectants that rely on their effectiveness in creating such a strong oxidative state often with hydroxyl radicals that together act to significantly reduce the microbial populations in the impacted environment.

To be effective, the disinfectant, as a minimum, has to reach the cell wall of the targeted microbial cell and either do irreparable damage to the cell wall or enter the cell and create irreversible damage. In order to achieve either of these impacts, the disinfectant has to penetrate through the bound water zone held in place by a complex web of polymers (that form the biofilms). Cationic disinfectants are likely to get absorbed into this matrix and never make it to the cell. In a growing biofilm, much of the volume is composed of the polymeric matrices with, commonly, less than 0.1% of that volume actually made up of microbial cells. The dispersed nature of the cells within the matrix thus makes disinfection more difficult to achieve. Often a dispersant and a penetrant are used concurrently in order to disperse these matrices and expose the cells to the action of the disinfectant. Given these limitations in effectiveness, there are also significant differences in the activity of the various chemical disinfectant treatments when applied to wells. These are summarized below.

D.5.1.1 Chlorine-Based Disinfectants

These include chlorine gas, chlorine dioxide, and forms of hypochlorite. The first two forms are very powerful disinfectants but require judicious use because of their extreme levels of chemical activity. Generally, chlorine gas use is restricted to carefully managed conditions while the chlorine dioxide can be applied more widely when used with an inhibitor to control the releases of the active chlorine dioxide. Most of the time it is the hypochlorites that are used either as the sodium salt (soluble) or the calcium salt (solid). Sodium hypochlorite is widely available in different strengths, commonly ranging from 5 to 12.5%, but degenerates significantly on storage. Application to a well site is convenient and low residual chlorine values in the low parts per million range can control the enteric bacteria. However, sodium hypochlorite is not as effective against maturing biofilms and the impact may be limited to a compression of the slimes that does improve conductivity in the short term. Once the hypochlorite has been flushed through the system and the residual value is gone, there is often a rapid rebound that again restricts conductivity through the infested region. In some wells that have been subjected to hypochlorite treatments, the short-term gain may only last from hours to a few weeks depending upon the severity of the fouling. Calcium hypochlorite has a much lower solubility and is often dispensed in a tablet or pellet form that dissolves slowly. There is some risk of these pellets/tablets hanging up and not dissolving completely causing concretions to form that could cause localized clogging.

Over the past four decades, disinfection using chlorine-based chemicals has lost a lot of its advantages due to the generation of potentially health threatening trihalomethanes (THMs) and possibly other compounds by interaction between the contaminants and the chlorine. In many states the use of these chemicals is severely restricted because of the risks posed by the THM products of the disinfection. There has been a swing to other disinfectants such as ozone. Disinfection is only one part of a treatment protocol to regenerate and its effect may be severely limited to some of the enteric bacteria (and in particular the coliforms) but not to the vast majority of the bacteria that are causing the biofouling.

D.5.1.2 Ozone

Over the last two decades ozone has been increasingly promoted as a replacement for chlorine-based compounds to achieve disinfection. Ozone is a very powerful oxidizing agent that creates an environment lethal to many microbes. This condition is enhanced by the releases of hydroxyl that can also act as bactericide. However, the effectiveness of the ozone is limited by the lack of any residual disinfectant activity. Thus even when a massive ozonation of a well is conducted, once completed, there would be no residual antimicrobial activity. Localized impacts with ozonation on the microbial populations can be dramatic, reducing the population often by more than four orders of magnitude. In an open system such as a well, the application of ozone would have a marked, but localized effect. The zone in which the microbial populations had been severely impacted would undergo a rapid recolonization by microbes moving in from outside the impact zone. These organisms would utilize the organic debris left from the treatment as a food source so that regrowth could be very fast.

D.5.1.3 Peroxides

Peroxides are also oxidizing agents and are lethal to microorganisms. However, peroxides are a normal biproduct of aerobic activity (respiration) and so microbes able to grow aerobically possess a biochemical protection mechanism. This mechanism is an enzyme system called catalase that rapidly breaks down the peroxides to water and oxygen. So universal is this enzyme that it is a part of a standard test to determine whether a bacteria is aerobic because it will usually be catalase positive. A good example of this in action is the application of hydrogen peroxide to an infected skin wound. Here the peroxide immediately erupts into foam caused by releases of oxygen through the action of the catalase. In practice, peroxides are rarely used as a single chemical treatment but more often as part of a blended chemical treatment.

Both ozone and peroxides create strongly oxidizing conditions with the releases of oxygen as a daughter product. The presence of this condition, along with the presence of oxygen, is likely to impact at least temporarily on the redox front moving it away from the treatment zone. At the same time, the saturation of the region with oxygen as a product of the treatment along with the residual organic debris left over from the treatment impact can stimulate very significant aerobic microbial activity. While the previous chemicals described above are relatively specific and easy to chemically define, there is also a large group of benzene- and/or phenol-based chemicals that do have significant bactericidal properties. They have been widely employed in the health- and food-related industries, but none have yet become widely adopted in the treatment of biofouling in wells. Generally, these chemicals are broad-spectrum disinfectants that commonly focus on the enteric bacteria and/or the Gram-positive cocci.

In the treatment of wells, the selection of a disinfectant as the treatment agent implies that the main concern would be health-related and that the objective is not to

effectively control the biofouling as such. In general, disinfectants have been designed to function most effectively on either flat relatively non-porous surfaces or in waters with a low to moderate turbidity. This effectiveness is compromised when attempting to disinfect porous media with the surfaces coated and the void spaces filled with microbial biofilms. In most cases where disinfectants are used they are used in combination with other chemical agents.

D.5.2 Acids

Acids are capable of breaking down both the polymers forming the slime and the matrices forming the microbial cell walls by a process called hydrolysis. In this process some of the bonds within the molecules are fractured causing the large molecules to collapse into fragments and so lead to the deconstruction of the biofilms and the death of the cells within those impacted structures. Hydrolysis usually becomes a significant event when the pH in the environment drops below 4.5 and can become extreme when the pH falls to less than 2.0. It should be remembered that dramatic as this may seem there are many microorganisms that can flourish in very acid pH conditions right down to a pH of 0.0. The effectiveness of an acid treatment is therefore partly dependent on the temporary pH swing created by the application of the acid. The temporary nature of the pH swing means that there would not be time for the acid-loving bacteria (acidophiles) to grow and become dominant. Effective pH swings from the ambient pH values should ideally be at least 3.5 pH units with greater than 5.0 pH units to achieve a maximum effect.

There are two major groups of acids, inorganic and organic. In general, it is the inorganic group that can achieve the greater pH swings but it is the organic group that sometimes has a greater disinfectant activity level. It is more important to achieve a designed pH swing towards an acidic state rather than to apply a specific quantity of the chemical. The pH meter and the determination of the extent of the pH swing become a more important measure than the weight or volume of the selected chemical.

It is often necessary to achieve the required pH shift to combine organic and inorganic acids to reach the regeneration goal. This combination results in an optimal detergent and disinfection capability.

D.5.2.1 Inorganic Acids

Inorganic acids commonly used in the regeneration of wells include hydrochloric (muriatic), sulfamic, and phosphoric acids. Each of these acids plays a very different role when applied and need to be considered separately.

D.5.2.1.1 Hydrochloric Acid

This has been directly used in the water well industry for many years as well as in many well cleaning chemicals. Third strength hydrochloric acid is commonly known as muriatic acid and this term is often preferred since this form of the acid is

known to be less corrosive than the concentrated and glacial forms of hydrochloric acid. The low pH generated by these acids is particularly effective against encrustations and carbonates. Camera-logging the well before and after treatment with the acid can show dramatic improvements such as screen slots now free from any forms of deposit or growth. There remains a risk that acid may also begin to attack the metal (such as stainless steel) and some formulations include additional chemicals to act as inhibitors. Usually the inhibitor added is a gelatin-based material that is able to control the aggressivity of the acid to the metal surfaces. Some of these inhibitors are toxic and so caution should be exercised when using inhibited acids. It should also be recognized that if the treatment chemicals are not completely removed from the treated well then there could be two possible concerns:

1. There is the effect of the residual nature of any toxic materials involved in the inhibitor.
2. Gelatin base applied with the inhibitor may act as an additional food substrate for the microorganisms recolonizing the impacted site.

In general, hydrochloric acids have been widely used in the industry with a direct impact on the inner surfaces of the borehole and screens. When used as a single source chemical treatment, these acids have not been found to be able to penetrate deeply into the packs and formations around the well. Some newer regeneration treatments do still use hydrochloric acid but only as one component in a blended treatment train. Caution should be exercised when working with these acids since they are very corrosive and all safety guidelines need to be observed.

D.5.2.1.2 Sulfamic Acid

Sulfamic acid is often used as a less vigorous form of acid treatment. This chemical will not drop the pH as effectively as hydrochloric acid but it is much easier to handle and can be purchased in a dry form. This makes transportation and handling much safer but gloves, masks, goggles and effective ventilation should be used. Sulfamic acid has been found to be particularly effective against carbonate-rich scales and encrustations but remains less effective against biofouling particularly where these are rich in metals. It is common for sulfamic acid to be used in blended mixtures with other acids.

D.5.2.1.3 Phosphorus-Based Acids

Another inorganic acid that has been widely used in the industry are forms of phosphoric acid. It is effective against the various metal and mineral hydroxides and has been used widely to remove concretions and encrustations rich in these materials. It is, however, less effective against biomass. There is a concern that treatments using phosphoric acid may tend to leave significant amounts of phosphate residues behind to stimulate microbial growth. Because of this risk of stimulating the reoccurrence of biofouling with these residues, the use of phosphoric acid and phosphorus-based compounds is NOT RECOMMENDED as a part of a regeneration strategy for extraction, injection, and horizontal wells.

D.5.2.2 Organic Acids

Organic acids most commonly used include oxalic, acetic, and hydroxyacetic (glycolic). These are described below.

D.5.2.2.1 Acetic Acid

Of the organic acids, acetic acid has had a history as a very effective biocide validated through the use of vinegar in the preservation of many foods. It has also been found to be very effective in the dispersing of biofilms. It is not as effective an acidizer as the inorganic acids described above and generally the pH can normally only be brought down to around 3.5. While acetic acid is widely available as a biproduct from many industries, it is strongly recommended that a food-grade or good industrial grade be applied. Some of the lower grades tend to have significant quantities of various metals and other compounds that might compromise the regeneration process. Acetic acid comes in various forms and concentrations ranging from 30 up to $>85\%$. There are two distinct problems associated with acetic acid:

1. Acetic acid tends to gel at temperatures below 13°C (55°F) making dispensing difficult if not impossible.
2. Strong odors are given off from the acetic acid that can become repulsive to the workers and inhabitants of regions surrounding the treatment site.

To combat some of these concerns, it is recommended that the acetic acid containers be kept at room temperature in regions where the ambient temperatures fall to less than 13°C (55°F).

Acetic acid performs as both a disinfectant that is very effective against many bacteria and also as an acidizer. Consequently, it is commonly used in the regeneration of wells. To improve the ability of acetic acid to act as an acidizer, sulfamic acid is often added to improve the ability to lower the pH down to less than 2.0. The odor problem can prove to be more challenging and this is best addressed by moving towards using hydroxyacetic (glycolic) acid in place of acetic acid. Glycolic acid is more expensive to purchase than acetic, but does offer the advantage of being odorless and safer to handle. To mitigate the cost increase over acetic acid, hydroxyacetic acid is a stronger acid and requires only one-third to one-half of the volume to effectively treat a well. Glycolic acid has replaced acetic acid as an organic acid application. This is because glycolic is easier to handle, does not smell, and is more effective as a chelator of iron than acetic acid. Commonly glycolic acid is supplemented with sulfamic acid to improve its effectiveness against iron-rich biomass where this is causing the biofouling.

D.5.2.2.2 Other Organic Acids

Other acids sometimes used are oxalic and citric acids. These acids are good acidizers in low-calcium waters and also act as chelators. However, both should be avoided in high calcium content (>125 mg/L) waters where it can generate insoluble precipitates that may induce additional clogging causing the regeneration treatment to fail. Citric acid is widely recognized to inhibit the activities of many

microorganisms and is a mainstay in the prevention of microbial spoilage in the beverage industry. However, citric acid does form a potential substrate for the growth of many bacteria in the sub-surface environment. This would mean that there would be a secondary risk that the citric acid would form a food substrate for many of the surviving microbes.

D.5.3 Dispersants and Penetrants

There is a wide range of compounds that have been generated as adjuncts to the detergent industry. These compounds fundamentally possess the ability to clean surfaces by a variety of mechanisms and are grouped according to the form of their effects on a biofouled region. There are many of these compounds that perform several different functions which makes defining a particular chemical difficult.

Where the effect is achieved by the compound penetrating into the biomass and causing disruption of the structures then these compounds are known as penetrants. Penetrants would be used under conditions where there is known heavy biofouling and the designated purpose is to enter into and disrupt the biomass causing break apart. They may also be used to break open pathways along which other chemicals can enter into the biofouled region and have a significant impact. On the other hand when the effect of the compound is to remove relatively thin layers of biomass from a surface, thus rendering that surface clean, the compounds are known as surfactants. For those compounds that are able to break up the biomass into sufficiently small particles that they move out into the water, these are considered to be dispersants. Essentially the penetrant enters into the biomass, the dispersants break up the biomass completely, and surfactants clean the surfaces.

Detergent has been the name given to those chemicals that are effective in removing foreign materials primarily from various forms of fabric. The detergent group tends to act more like surfactants in that the action is essentially surface cleaning. There is a considerable functional overlap between these various chemicals that is further complicated by the fact that these compounds may be anionic, cationic, or neutral in their charge. The form of this charge clearly would influence the manner in which the applied chemical compound would function as a penetrant, surfactant, detergent, or dispersant.

In the marketplace, these chemicals have achieved considerable use as synergistic stimulators of other chemical activity (such as through improving the effectiveness of pesticides and cleaning agents). This gives these products considerable value and their formulations and precise abilities are protected as commercially confidential. As a result the exact natures of the various chemicals in this group are often difficult to determine but their adjunct effects on various chemical processes is well recognized.

It is rare for these compounds to be used as a sole treatment chemical since their addition is usually associated with improving the efficiency of some other chemical treatment. There is a growing group of these compounds that have additional functional ability that may allow them to be used as a sole treatment agent. For example, the bacteriostatic detergent, CB-4, an anionic polymer, is not only an

effective dispersant of biofilms at concentrations above 0.01% but is also a biocidal at concentrations in excess of 0.5%. Thus this compound has merit in the preventative servicing treatment since it is not only able to disperse biofilms but also able to kill many of the incumbent microorganisms. As research progresses, it is probable that there will be more use of these compounds in the control of biofouling post-radical treatments.

D.5.4 Alkalies

Acid treatments have long been recognized as a suitable means for reducing the impact of clogging and plugging on wells. The use of alkaline treatments, taking the pH up, has not received the same recognition. While it is known that alkaline conditions, particularly at pH values above 9.5, are known to be inhibitory to many microorganisms, the impacts of raising the pH upwards (instead of lowered as in acid treatments) does not bring about the same degree of lethality. In other words, raising the pH from 7.5 (close to neutral) to greater than 11.5 (a 4 pH unit shift upwards) does not yield the same dramatic reductions in microbial numbers as acid treatment would taking the pH down 4 units. The reason for this is twofold:

1. As the pH goes higher the bound water (slime) polymeric matrices around the cells in the biofilms tend to thicken to provide additional protection from the pH impact.
2. Elevated pH can cause carbonate and metal precipitation and the formation of concretious structures that now also shield the surviving microbes.

As a result of the described limitations in the effects of alkaline treatments, it has never been widely adopted by the industry. In laboratory studies, it has been noted that following an acid treatment (to lower the pH) with an alkaline treatment (to raise the pH above the original pH of the water) can result in a pH shift of 7.0 or greater pH units. The additional pH-induced stress has been found in both laboratory and field studies to increase the effectiveness of the regeneration process on the wells. This combined treatment is referred to as the "flip-flop" treatment since it flips the pH one way and then it flops the other way.

The two alkalies commonly used for such treatments are sodium and potassium hydroxides and both are easily capable of raising the pH of the water up to as high as pH 11.5. The sodium form tends to be the more aggressive and should be used with extreme caution if there is a significant amount of clay in the formation materials around the wells. The sodium alkalis can cause the clay to swell causing flow into or out of the well to be partially or completely blocked. It should be remembered that if a "flip-flop" has been planned after an acid treatment, there should be a "buffer" of water injected into the well in order to keep the acidic and alkaline elements apart during the treatment. Mixing acids and alkalis can cause eruptions or explosions. The volume of the buffering water between the two treatments should be a minimum equivalent to three well volumes.

D.5.5 Selected Blended Mixtures of Chemicals

In the water well regeneration industry, the bulk of the chemical treatments are blended thus involving more than one active chemical ingredient. The exact formulations are commonly proprietary and cannot be easily obtained. It is recommended that the materials data safety sheets be obtained and carefully examined for possible undesirable impacts. There is a range of typical combinations that are widely used. Many such formulations include a proprietary penetrant, surfactant, dispersant, detergent (PSDD) to facilitate the effectiveness of the treatment. Such a proprietary chemical would be incorporated with one or two other chemicals in a solid or liquid form. Inhibitors to reduce the speed of a given reaction may also be included. The nature of the blended chemicals and the form of the treatment should closely follow the manufacturers' guidelines in order to ensure the potential effectiveness of the treatment.

Typical combinations found in the marketplace include, but not limited to, the following broad groups:

1. Organic acids with a PSDD.
2. Peroxides with inorganic acids and PSDD.
3. Inorganic acids with a pH color indicator to ensure an adequate drop in pH is achieved.
4. Inorganic acids with PSDD.
5. Disinfectants commonly with some pH modifier and PSDD.

The proprietary nature of these blended products and the lack of comprehensive independent trials to determine the effectiveness of these products limits the validity of the recommendation of any particular product. In general, there is a considerable variation in both the treatment costs and the validation/assurance procedures required for validating that the products are actually effective. It is recommended that local effects of chemical applications that have been validated from the application should be reviewed before deciding on the choice of an applicable product.

D.6 OVERVIEW OF TREATMENTS BLENDING CHEMICAL AND PHYSICAL METHODS

The varied nature of many fouling events involving growths and elements of clogging, from the borehole out into the formation, often involves the need to maximize treatment effectiveness by including a more comprehensive blend of both chemical and physical elements. While these technologies are generally more expensive to apply, they often can be more effective in controlling the fouling event. This may simply be because the disruption and dispersion of the fouling is more effectively tackled using a comprehensive blending of physical and chemical treatments. Of these blended forms of treatment, the following are in use: pressurized injection of carbon dioxide and blended chemical and heat treatments.

D.6.1 Pressurized Injection of Carbon Dioxide

Carbon dioxide, like citric acid, has long been used in the beverage industry as a chemical that can suppress biological activity. While citric acid, as an organic acid treatment for wells, has largely been discarded because of the secondary bacterial growths that can occur from microbes feeding on the citric acid, carbon dioxide applications have increased. The injection of carbon dioxide can act to inhibit and remove biofilms and biofouling from wells through a combination that can include freezing, the generation of carbonic acids, and a surge in pressures within the biomass formation and the well. When carbon dioxide is injected at very low temperatures into the biofouled zones in and around a well, the temperatures in those zones will also drop, leading to some of the bound water within the biofilms freezing. As the ice crystals form within the biomass they can also form at the attachment sites of the biofilm to underpinning surfaces. This activity causes the biofilms to be lifted up from the attached surfaces and be disrupted. The net effect is that the biofouling structures are shattered and dispersed into the freezing water within the voids. As the carbon dioxide reacts with the groundwater, carbonic acids are formed that reduce the pH of the environment which can also inhibit and kill many of the microorganisms that survived the disruption of the biofilms involved in the biofouling. Like the freezing, this action is also transient and will be neutralized as new groundwater enters the treated zone. The impact of pressurized carbon dioxide treatment involves the application of significant pressures to force the carbon dioxide into the formation. In general the microbial kingdom has a much greater tolerance of pressure changes than plants or animals and the types of pressures applied may not significantly increase the lethality inherent in the treatment.

Application of carbon dioxide can range from the simple act of dropping solid carbon dioxide (dry ice) pellets down the borehole to sophisticated patented application techniques in which large volumes of carbon dioxide are injected down the borehole under pressure. Like many other well regeneration treatments, this treatment involves the movement of the treatment chemical out into the formation and is also likely to take the hydraulic lines of least resistance in moving away from the application site. Movement of the chemicals out into the formation would mean that the effectiveness of a treatment may be witnessed by carbon dioxide appearing at a well 100 yards or more away from the well being treated. In reality, this may be a reflection of the probability that there was a pathway of least resistance between the treated well and the well where the carbon dioxide gas appeared. That does not necessarily mean that all of the biofouling around the treated well has been impacted or removed but simply that a path had been established in the biofouling in the direction of the well now showing the presence of carbon dioxide. Field experience has shown that there have been successful treatments in consolidated wells in hard rock fed by fractures. Here, the limited pathways created by the fractures and open structures around the borehole would be the sites of the biofouling. As the carbon dioxide comes into contact with most, if not all, of these biofilms and deposits associated with losses in production for the well, then the biomass can be severely disrupted.

The application of freezing and pressures to a well does pose three possible risks and concerns

1. When water freezes into ice there is an expansion in the volume that could put additional pressures on the structures within the well leading to well structural failure;
2. Application of pressurized carbon dioxide down hole has to be done under carefully controlled conditions to ensure that either the injection apparatus is not forcibly expelled from the well or that the pressure does not collapse any of the installed well structures;
3. Formation types may pose a major risk and concern to the application of pressures particularly in an unconsolidated formation in which movement may be significantly affected by the small and fragile nature of vulnerable pathways. Consolidated formations (such as bed rocks) pose less risk of collapse and an increasing probability that the treatments will be propelled by the applied pressures along fractures in the rock. This would achieve greater penetration but in only the directions of least resistance to the applied pressures.

D.6.2 Blended Chemical and Heat Treatments

Application of heat as a sole treatment to recover wells from biofouling has been attempted periodically over the last century. Practices through 1970–1985 showed that heat alone often not only killed off the majority of the microbial population but also caused a seemingly non-reversible coagulation within the biomass creating a concretious plug that became almost impossible to open again. In the last 15 years, attempts have been made to combine the clear and obvious advantages of heat to control the microbial populations with chemical techniques to not only prevent concretions from forming but also to aid in the effective removal of the various elements causing the fouling. United States Army Corp of Engineers (USACE) investigated a process combining heat and chemicals through the Repair, Evaluation, Maintenance and Repair Program at the Waterways Experiment Station in Vicksburg Mississippi in the 1980s and 1990s. A commercial patented application of heat and chemicals has been successfully applied to a number of USACE sites ranging from relief wells at levees and dams to injection and extraction wells at groundwater remediation sites. The types of acids and the heat generating equipment used have evolved over time and the process was successfully used to rehabilitate extraction and injection wells at several Comprehensive Environmental Response Liability Act sites.

The major benefits of blending many methods into a treatment stream is that, when properly applied, the advantages of the chemical are enhanced in a synergistic manner particularly when the heat speeds up the rate at which the reactions occur. Taking the temperature up by 10°C (18°F) is generally thought to have a one order of magnitude increase in the rate of that chemical reaction. By applying heat during the addition of the PDSS, disinfectants, and acids or alkaline treatments then the

reactions (reflected in the collapse/disruption of the biomass) occur that much quicker.

The net outcome of this approach to regeneration is that there would be a thermal gradient extending away from the application site (e.g., the borehole). While these temperatures would be lethal to most microbes infesting regions close to the borehole, there is a thermal gradient moving out from the well. This could mean that at distances of perhaps a meter (or a yard) the thermal gradient would fall into the range where there could be enhanced microbial activity. This is not a significant potential since the thermal gradient dissipates quickly at the end of the thermal stage of the treatment. There is not enough time for the traumatized microbial survivors to adapt to these high temperatures and grow effectively. It has to be remembered that in the effective regeneration of a biofouled well, the maximum effect of the chemical and heat applications has be performed in concert with the mechanical methods that are intrinsically a part of the shock, disrupt, and disperse treatment cycle. These three phases in the blended chemical heat treatment (BCHT) of plugging water wells are describe below:

SHOCK phase is designed to disturb the nature of the biofouling elements by the combined application of heat (to traumatize and kill many of the incumbent microbes) along with a disinfectant to further improve the lethality of the treatment. At the end of this phase, it would be expected that most of the microbial cells would be minimally traumatized by heat and many killed by the disinfectant action. These effects are heightened by the use of an appropriate PSDD.

DISRUPT phase is next to begin the process of destroying the integrity of the biofouling elements by disrupting the structural integrity of the biomass. The heat input is maintained but now a radical shift in pH upwards or downwards by at least 3 pH units or a flip-flop to get a 7 pH unit shift is applied. At the same time a PSDD is used to penetrate the collapsing structures and cause further disruption.

DISPERSE is the final phase of the Blended Chemical Heat Treatment regeneration treatment causes the structures that created the biofouling to shatter but still occupy pore spaces in fractures, the well screen, the gravel pack, and the adjoining aquifer. The dispersion phase continues while the PSDD treatment further collapses structures and the temperature is allowed to decline. Pressure- or air-lift surging is used to move the particles out of the well through the borehole or force the particle deeper into the environment surrounding the well where there would be less direct impact on the future operation of the well.

D.7 CHEMICAL TREATMENTS: TRADITIONAL VERSUS NEW

There has been a traditional attitude that treatment of any sort of geochemical or biological problem can be addressed by a single-sourced chemical treatment. In the

medical industry, this thinking has led to a very powerful development of chemicals designed to target specific problems.

To address these problems, a range of chemical treatments have been developed over the years that, unfortunately, have been found through experience to not address all of the challenges of the extraction, injection and monitoring wells. Diagnostic techniques were limited in their application and not designed to adequately determine the root causes of the symptoms observed (i.e., in the biomass). The uncertain nature of the chemical treatment in some cases coupled with the desire to replace wells rather than rehabilitate them has led to a poor understanding of the mechanisms involved.

Traditional treatments, although practiced over the last 50 years, have not generated a level of confidence that they effectively addressed the problems. The new generation of treatments coming into the marketplace as verifiable technologies generally recognizes the broad spectrum of problems that can occur in wells. It is for this reason that most of the newer treatments accept the need to apply more than one chemical therapy and/or improve the effectiveness of a physical treatment process. At this time, the quality assurance and quality control (QAQC) are not clearly specified and there are no base line compliance requirements. As the understanding of the nature of clogging and plugging in wells on contaminated groundwater sites becomes better understood then QAQC compliance guidelines would become a part of the treatment practice. General points to consider are listed below in the election of a treatment option.

D.7.1 Chemical Treatments of Wells

These treatment techniques have been practiced for more than a century. While there are many different proprietary products in the marketplace, only limited attempts have been made to conduct an independent evaluation of these technologies. Any evaluation immediately faces the task of making comparisons in treatment effectiveness for a wide variety of products, each of which may function very effectively under some circumstance and fail to achieve any effects on others.

D.7.2 Traditional Technologies

These have used some relatively simplistic approaches that involve a single chemical strategy designed to treat a particular problem such as iron encrustations, carbonates, slimes, and taste and odors problems. Typically, acids would be used to control carbonates and iron encrustations while disinfectants would be used to treat slimes, taste, and odor problems. In the last 30 years there has been a movement to include various PSDD to improve penetration of the active chemicals. Modern products usually are blended mixtures that are claimed to have a greater effectiveness within a particular set of conditions. There remains a reluctance to increase the effectiveness of chemical treatments by heating the well up to accelerate the chemical action.

D.7.3 Chemical Treatments in Preventive Mode

A major aspect of well maintenance and fluid system performance is the ongoing maintenance of wells. Redevelopment is usually needed to improve the well's effectiveness. Experience shows that chemical choices in well treatment are often made based on incomplete information and/or the claims in the vendor sales literature. While information should not be dismissed if it comes from a commercial source (as vendors frequently seek to educate), it is crucial that personnel engaged in the planning the operations and management practices for a new well system should seek expert advice and review publications. Publications specifically written for these types of sites are a necessity for the operator to become well acquainted with the features of chemical choices, both for effectiveness and safety.

It should be remembered that some chemicals present a range of risks to the operators and all of the standard guidelines for the health and safety of all operators at the treatment site need to be followed. Remember that even when a treatment has been successful in regenerating a well, the dispersion phase will cause spent chemical and disrupted biomass to be discharged from the well. Care has to be exercised particularly when that discharge is very acidic or alkaline. This would mean that the discharge would need to be neutralized so that it does not impact at site on the natural environments. Additionally, the disrupted and dispersed biomass may contain very high concentrations of chemical accumulates (such as metals and recalcitrant organics) that may now require safe disposal as a potentially hazardous waste. Local regulations commonly set the guidelines for what may, or may not, be required when the discharge is hazardous. It should be remembered that the water well, in operating as a natural filter, would cause these types of accumulations and at the same time have produced better quality waters because of this filtration.

D.8 SELECTING WELL VOLUME TO TREAT

One challenge is the determination of the amount of formation material around the well that would be treated at the same time as the borehole is being treated (see Table D.1). One well volume would be expected to treat the water in the borehole and perhaps the slots, perforations, or fractures in intimate contact with the borehole but not more. One bore well volume is therefore only suitable when there is to be a preventative maintenance applied or rehabilitation where it is known that the biofouling is limited to the borehole itself. If the biofouling extends back into the porous media and formation material around the borehole then greater than one well volume would need to be applied. The number of well volumes that can be applied can range from one to six.

One of the major problems in the regeneration of water wells is the tendency for complacency since water wells commonly function over years and decades and changes are commonly subtle. Past practices have been to abandon and replace. This creates economic activity and an assurance that the new well will now function will function just like the old well when it was first developed. This is not always the case particularly for those who do not recognize that the immediate environment around

TABLE D.1
Selection Table for the Number of Well Volumes to be Used in Well Regeneration and Preventative Servicing

Well Volume	Treatment Type	Comments
1	Preventative maintenance (PM) level one	May also be applicable if there is a high degree of certainty that the biofouling is limited to the borehole environment only
2	PM level two or mild regeneration treatment	If the Q/s has declined within the 5%–20% range and the zones of interrogation projections (ZIP) shows bacterial activity is still local to the borehole environment
3	Regeneration level three	If the Q/s has declined within the 20%–40% range and the ZIP and other information confirm that the biofouling is focused close to the well this would be considered effective
4	Regeneration level four	If the Q/s has declined within the 20%–40% range and the ZIP and other information confirm that the biofouling is focused further out from the well this would be considered effective
5	Regeneration level five	If the Q/s has declined within the 30%–50% range and the ZIP and other information confirm that the biofouling is focused further out from the well this would be considered effective
6	Radical regeneration level six	If the Q/s has been reduced by greater than 40% and there is evidence of very active bacterial fouling then this should effectively reach out into the far reaches of the biomass and cause disruption

Note: Well volumes are cumulative and can include the treatment volumes used for shock, disruption and dispersion. In the event that the two phases of the treatment involves different chemicals that can react if they come into contact (e.g., acids and alkalies) then an additional well volume of water should be included to act as a buffer to prevent violent interactions.

abandoned water wells because biofouling is heavily infested with the microbes associated with the plugging of the original well. Drilling in a new well close to the abandoned well virtually guarantees that the new well will be taken over by the microbes left behind from the abandoned well. When installed within a zone already biofouled then the life span of the new well will be negatively impacted and a shorter operating life is an inevitable consequence. While that might be good for the consulting engineers and the well drillers it is not good for the economical and sustainable operation of the new well. Positioning a new well within a field known to

include historically known to have wells that were biofouled is another art and to place a minimum distance between old wells and the new wells to be constructed. That is another art form. Basic guidelines would include using the maximum distance of convenience, moving upstream within the aquifer, examining the Q/s and zones of interrogation projections (ZIP) data along with any other information to determine the best location which will almost inevitably not be the most economical (from the accountants point of view) or the most convenient (from the plumbers and planners points of views). That is why the art of well development is so important since it is essential to build and maintain well fields that offer confidence that a sufficient volume of water will be produced of an acceptable quality to comfortably meet demands.

> In the case of iron-bacteria, however, the problem is complicated by the fact that these organisms are endowed with a capacity for absorbing large quantities of organic to the molecules of which iron is attached, and with the possession of a sheath which is capable of retaining the iron that is liberated to the outside after the organisms have done with the organic matter to which it was originally attached. (Ellis, D., 1919, *Iron Bacteria*. London, Methuen & Co. Ltd, 125.)

E Sustainability of Water Wells

E.1 INTRODUCTION

There is a history and a future for every well drilled into the ground. Knowledge of that history and parallel experiences with other wells can allow for better management, a longer operating life (making the well more sustainable), and finally allow a safer closure and abandonment at the end of its life. This chapter covers the methods to make water wells more sustainable through applying the proper preventative servicing and on-time regeneration.

As the economic conditions tighten and durability becomes a bigger concern it means, for the water well, that it has to move from being viewed as a disposable item that is abandoned when it fails to becoming a durable item that will last much longer. Sustainability means to prolong the active life span for the well through vigilant maintenance servicing and prompt positive reactions to the problems as they inevitably emerge.

E.2 DEFINING SUSTAINABILITY

Water wells fail for a combination of reasons such as equipment failure due to corrosion or wear, ongoing faulting and shearing in the geologic formations, dewatering of the aquifer, clogging, and/or plugging in, and around, the well (affecting the specific capacity, Q/s), changes in the biology and chemistry of the water (leads to failures in water quality); and the admission to the well of groundwaters from alien aquifers (causing radical changes in the water quality and quantity produced by the well). Some of these factors are difficult to control. Other factors are less controllable where these relate to the aquifer dewatering or the geologic formations becoming unstable. Sustainability addresses those issues that are controllable through diligent management practices. The prime objective is to extend the active life span of the water well from one which is simply pumped until it fails and is then abandoned to one that is serviced to maintain the Q/s, keep the water quality acceptable and ensure the longest pay-back time.

In the Canadian prairies, water wells all have a definable life span from development into a producing well to final abandonment. This life span varies across the three prairie provinces but an average life expectancy for a water well today is approximately 15 years with some lasting twice or three times that long while others

fail in less than a couple of years. This variation is, in part, the effect of aquifers delivering groundwater of different qualities to then pose a different set of threats to the well. This is because each well has a unique environment with different geologic strata outside and various forms of well construction inside.

Sustainability would dictate that the average life expectancy of the water well should be increased through better management practices, recognizing that it is no longer an option to neglect the water well and abandon it as soon as it fails. We are running out of space to reasonably locate these wells and running out of groundwater resources, which mean that we have to harvest the water with a view to the needs of the future. There are parallels between the operation of a water well and the operation of an automobile. For the automobile, it is understood that the gas tank has to be filled when it empties, the oil has to be changed in the engine, tires have to be changed when they are worn, and replace the battery before it fails. We do this to maintain the automobile because it will then become dependable and less likely to leave us stranded in the middle of nowhere on the way to somewhere. A water well is in some ways like the Energizer® battery bunny that is always ticking on, and on, and on. Water wells do not normally come with a guarantee from Nature to continue to pump water for so many years without changes. Given that there will have to be some maintenance of the water well over and above the failure of equipment through normal wear and tear events that have to be replaced, there is also a need to develop a strategy that would convert neglected wells into sustainable wells. That decision would mean diligence in ensuring that each well is functioning effectively and a willingness to spend the effort and funds to ensure that the well will just keep on ticking. As the well ages then it will be subjected to both support and attack by the microorganisms infesting that well. Support is achieved by the microbes forming the natural filters and improving the quality of the water. The attack phase is more complicated involving the biomass growing to the point of plugging, maturing to the point that it begins to shed its "dandruff" (biocolloids rich in accumulated metals and recalcitrant organics), creating unpleasant odors (often experienced with maturing biomass), and starting corrosive processes using hydrogen sulfide or organic acids as the means to penetrate into the metals and concretes used in, and around, wells.

Two major symptoms come into play to determine the health of the water well. For us humans those primary health features are body temperature and blood pressure. For water wells the parallel events are a little more subtle:

1. Production performance (perhaps the equivalent of the blood pressure).
2. Changes in the water quality that could related to rampant infestations down in the well (perhaps paralleling what we use the body temperature for, to determine whether we have an infestation!).

As there are a number of data-generating parameters that can be associable with the infestation of the well this data needs to be examined forensically for critical signals of impending failure. These two parameters (production performance and quality forensics) can be used to generate the warning signals that the well is going

to require some attention/treatment to maintain the quality management of the well. These factors are discussed in more detail below.

E.3 PRODUCTION PERFORMANCE

The prime measurement for well performance is the Q/s of the well. This measures the average volume of water being pumped from the well in a fixed time interval over a defined vertical distance below the static water head in the well. This measurement forms an excellent method for determining the ability of the well to produce water. This Q/s is affected by many factors, some of which can be directly related to the loss in flow potential into the bore hole because of these plugging and clogging events occurring in the near locations of the formation influenced by the pumping. Effects of this loss in immediate flow capacity are that the cone of compression during pumping becomes larger and goes deeper as it draws water from a greater area. At the same time, the water level during pumping will draw down in the water column to a greater extent in order for the pump to deliver a similar volume of water. This would cause the Q/s to fall. A prime objective in the sustainability of a water well is to prevent significant declines in the Q/s through preventative maintenance (PM) servicing programs.

One of the major problems in using the Q/s is finding an original Q/s value that truly reflects the original status of the freshly developed well. There are a number of possible constraints that could impact on the value of the Q/s when the well has been first developed:

1. Improperly developed well is likely to still have, in the immediate formations and porous media around the well, perched inert materials such as sands, silts, and clays which could negatively affect the Q/s until they are freed up by the pumping action and groundwater flow patterns when the well comes into full production. If this has happened then the Q/s value will tend to be low.
2. Newly developed well is presenting a virtual plethora of new environments within the forming redox front where microbial attachment and colonization can occur. These surfaces would become infested with rapidly expanding growths primarily in the forms of biofilms which interconnect. During the early stages in this infestation these biofilms can occupy a very significant part of the void and/or fracture volume (e.g., 20%–50%) during a radical expansion phase that commonly follows initial attachment. This would cause the Q/s to fall significantly and become highly variable during development.
3. Expanding biomass that has attached to the new surfaces within the redox front now goes through a phase of radical compression to occupy less than 2% of the available void and fracture volumes within the affected regimes. In compressing, the surfaces of the expanding biofilm become very smooth allowing even faster flow rates over the surface than the original uninfested surfaces had! This would mean that the Q/s would now rise to higher values than when the biomass growth finally stabilizes.

4. Final stage in the initial development of the well would be reached when all of the perched fines that could dislodge have been removed by flow action; the biomass itself would now have stabilized and entered a slow harmonic state of phased growths.

At this time, the Q/s would be precise and stable indicating that the well has been fully developed. It is this Q/s that should be used to determine when preventative servicing or regeneration should be undertaken on the well. Clearly a premature Q/s taken during phases 1, 2, and 3 in the development would give falsely low or high values.

The bottom line for using the Q/s is that this data can be used in the same manner as taking a blood pressure test in patients. The ideal would be to keep the Q/s at 100% but this is not likely to happen if there has been plugging/clogging in the well environment or if the aquifer is dewatering. If there is ongoing dewatering then this is going to affect the static water level in other wells, piezometers and monitoring wells. If the static water level remains stable then it can be taken that the losses in Q/s are a result of clogging and plugging events that are active in, and around, the well. Ideally, there should be a zero tolerance to any losses in Q/s by water wells. However, it is a time-intensive effort to obtain an accurate Q/s and it is highly likely that, even in a perfect world, there could be significant declines in Q/s between the two tests being applied. This means that, like a physician taking regular blood pressure tests for a patient at risk, routine Q/s testing should be applied to production water wells on a regular basis. Regular, as a word, can have different meaning depending upon the perceived risk. In the twentieth century, when water wells tended to be viewed as disposable items requiring minimum maintenance, Q/s may only have been undertaken some time during the development of the well. In the twenty-first century, where the attitude is now changing towards sustainability, there is a need to ensure a longer life span for the wells. One prime indicator of this is maintaining the Q/s at close to the original level as possible and having a zero tolerance for any drops in Q/s of greater than 5%.

Reaction scenarios of falling Q/s may be summarized as:

1. Zero tolerance for any losses in Q/s exceeding a 5% decline.
2. Target of maintaining the Q/s of the well at 100% by routine PM servicing practices that have been customized to meet the needs and to ensure recovery.
3. Generate a target that the well would never fall to less than 80% of the original Q/s (a loss of 20% in Q/s) without implementing a full regeneration program to restore the well to its full production potential.

These reaction scenarios make the assumption that there are adequate funds for ongoing maintenance of the well. Unfortunately, there are occasions when maintenance funds can be raided to meet other needs which mean that the maintenance just is not done and the Q/s could slide further down.

As the Q/s declines, there is a diminishing probability that the well can be returned back to its original state. Additionally as the Q/s declines then both the costs to achieve recovery go up and the probability of success now goes down. Regeneration has much more significant cost inputs than a PM servicing treatment and so the bottom line has to be to keep the PM servicing going and be prepared for this to occasionally fail which should mean that a fast tracked, appropriate regeneration is undertaken.

Triggers for the automatic enactment of a PM servicing should be a loss of no more than 10% of Q/s, but preferably there should be a PM even if the loss is only 2%–5%. Preventative maintenance treatment may be considered to have failed if the post-treatment Q/s (taken no sooner than 6 weeks after the treatment is applied) does not recover towards the original Q/s. This failure may be an occasional occurrence and should enact a second PM treatment. If this now fails to recover the Q/s then it can be expected that the well will continue to degenerate.

Triggering a regeneration of the well should occur when the Q/s has degenerated by greater than 20% (80% of original Q/s). If the well is left to continue to degrade then the potential for a full recovery to original specifications goes down. Generally the "gray zone" where a Q/s can be returned by regeneration to original becomes less achievable as the Q/s continues to fall. For wells that have lost greater than 40% of the original Q/s then there is a lowered probability for full recovery. If the well has lost 40%–60% of the original capacity, then there remains a potential for full recovery provided that the regeneration is appropriate to the wells problems and vigorous in its application. In cases where the well has lost greater than 60% of the original capacity then it is much less likely that the original Q/s can be attained. Generally under these circumstances, a regeneration of a well could be deemed to have been effective if the Q/s recovers by 40%. That would mean that a well that had lost 60% of its original Q/s would, through treatment, have recovered 40% to be now at 80% of its original performance. Wells that have lost greater than 60% of the original Q/s may generally not be able to be rehabilitate so successfully. This would be in part because the entrenched plugging and/or clogging can no longer be so effectively removed from the impacted well. Recoveries can be expected to be in the 10%–30% towards the original Q/s which may prolong the useful production life of the well.

Some practitioners in well PM servicing and regeneration tend not to rely upon the original Q/s as the benchmark but take the gain in Q/s from before treatment as being the indicator of success. For example, take a well that has lost 80% of its Q/s and subjected to a treatment that causes a 20% improvement. It can be interpreted as a treatment that has doubled the Q/s of the well and has given a 100% increase in the Q/s. While this is mathematically correct using the data limited to the pre- and post-treatment analysis of Q/s; it really represents only a 20% return towards the original Q/s which is still 60% less than the original Q/s for that well. Improvements of perhaps "100%" or even "200 + % have little meaning in this case except to acknowledge that the well was badly fouled and that the treatment did have some significant impact on the Q/s. All well treatment evaluations should use the original Q/s for that specific well as a defensible benchmark.

E.4 QUALITY FORENSICS

In addition to the use of Q/s as an indicator for a failing well due to fouling, there are other indicators that can also be relevant. Given that the cost of performing routine Q/s can become excessive then it can be that a monitoring program to assure sustainability for the well includes some water quality parameters that can also indicate when a well is beginning to lose its Q/s and fail. These parameters relate to changes in the water quality characterization that would occur if plugging and/or clogging is happening. Losses in Q/s for the well mean that there is already a train of failures occurring in the well that could include any, some, or all of the following:

1. Shortening time lapses as activities increase for specific groups of bacteria involved in the fouling become more active.
2. Increases in the iron content in the pumped water from the well as the bioaccumulation function in the biomass begins to fail.
3. Increases in turbidity (cloudiness) in the pumped water as biomass begins to sheer away and be carried in the groundwater to the well.
4. Changes in the dominant bacterial types as the biomass undergoes maturation.

Each of these potential symptoms of failure is addressed below. It should be recognized that for purposes of determining potential losses in Q/s, the role of plugging and clogging both involve microbial factors. In plugging, the microbial activities dominate the process because they are the major contributors to ongoing losses of void and fracture volume capacity around the well associated with biomass growth. In clogging, the initial problems for the well are created by the perching of inert materials such as sands, silts, and clays (fines) in the producing zone around the well. These perched materials create an environment where there can now be rapid microbial infestation. This means that in the event of a primary clogging due to the perching of fines there is a probability that a microbial biomass will be generated causing slimes and possible concretions to form. Thus, in the event of plugging there would have been the generation of a biomass already while with the clogging the biomass would be generated after the start of flow restrictions created by the fines.

Shortening time lapses using biologic activity reaction test (BART) is one common early feature for wells that are becoming plugged or clogged. This indicates increasing biologic activity. As the biomass in the well is becoming more active at least some of the bacterial groups infesting the well should also show greater activity. When a well begins to biofoul with the onset of significant plugging, the product water will gradually be carrying increasing populations of microbes that, primarily, have sloughed from the biomass into the flowing groundwater. As this plugging/clogging increasingly affects the Q/s then so the activity of the microbes within the water also increases. This directly causes the time lapses in BART testers to get shorter for those microbial groups. As the time lapses shorten that means the bacteria group is becoming more active (aggressive) and posing a greater risk to the functional integrity of the well (Table E.1).

TABLE E.1
Significant Time Lapse Shifts (Days) for Major Bacterial Groups Using the BART Tester Methodologies

BART Types	Background	Low Significance	Moderate Significance	High Significance
Sulfate-reducing bacteria	>10	9–7	6–3	<3
Iron-related bacteria	>8	8–6	5–4	<3
Slime-forming bacteria	>6	6–4	3–2	<2
Heterotrophic aerobic bacteria	>4	4–3	2	<2
Denitrifying bacteria	>5	5	4	<4

Note: when the time lapse for any of these tests moves across the border to a level of significant activities (i.e., moving along the row from one column to the next one on the right), then the level of concern relating to microbially influenced plugging and/or clogging would increase. If the time lapses enter the high significance (right-hand column) then there is a very strong probability that the well Q/s would have been impacted by these microbial activities. Background refers to the typical activities that would be expected to occur in natural groundwaters not stimulated in an active manner by the producing well. Note also this table uses a baseline generated for room temperature and these time lapses would be shorter or longer if different temperature were employed.

Each BART tester detects specific communities of bacteria and each of these groups has very different time lapses and reactions that could be construed as significant. There would be differences in priority for the different bacterial groups to signify that the well is biofouling. For the sulfur-reducing bacteria (SRB), there would be acceleration in activity and shorter time lapses where the groundwater contained higher sulfates was reductive, and the organics were dominated by short-chained fatty acid (reduced forms of organics that can be degraded by the SRB). For the iron-related bacteria (IRB), the triggering of this bacterial activity is oxidative conditions in which there is a significant amount of iron present in the groundwater. This iron can be in the form of either ferric (oxidized) or ferrous (reduced), and the concentrations that can stimulate the IRB range upwards from 0.1 ppm (minimal effect) to greater than 1.5 ppm where there can be extensive activity. There appears to be changes in the dominant type of IRB that is controlled by the relationship of iron to manganese with common rations centering around: 100:1, 20:1, 1:1, and 1:100. Iron-related bacteria activity is influenced by the amount of available organics with total organic carbon (TOC) levels in the range of 1–10 ppm appearing optimal. Where the TOC levels exceed 5 ppm, there is now an increased competition from the slime-forming bacteria (SLYM) and heterotrophic aerobic bacteria (HAB) communities, and where HAB are dominated by pseudomonad bacteria, the IRB can sometime be dominated by the slime-forming IRB. Slime-forming bacteria activity

is dominated by those bacteria that are very active in the redox front and generate a copious amount of slime during growth which can cause rapid plugging and bio-accelerated clogging.

Bacteria that are more active in an oxidative regime than in a reductivecondition are generally more adaptable to a wide variety of conditions. Organic carbon is important for the formation of these slimes and typically 5–10 ppm of TOC in the groundwater is sufficient to stimulate extensive formations of slimes. Heterotrophic aerobic bacteria differ from the SLYM, IRB, and SRB in that they are narrow spectrum degraders of organics under mostly oxidative conditions. This narrow spectrum means that they will tend to dominate under conditions where there is a limited range of different organics but in relatively high concentrations (e.g., $>$ 50 ppm) such as the petroleum hydrocarbons formed into a plume that then interacts with the well microflora. Bioremediation processes that are commonly oxidative rely heavily on the degradative activities of these bacteria to achieve removal of chemicals of concern. Denitrifying bacteria (DN) are the last group of bacteria that can be found commonly at the redox front or on the reductive side of that front. These bacteria are more specialized in function and can reduce nitrates to dinitrogen gas in the presence of significant organic carbon. Generally, these bacteria operate downstream of sites where there has been an impact on the groundwater by organic material rich in nitrogenous material (e.g., septic tank waste). Here some of the nitrogenous organics are reduced to ammonium as the common end product. When that ammonium enters an oxidative (oxygen rich) environment, it is attacked by nitrifying bacteria with nitrates being a principle end product. If the groundwater is now rich in nitrate and organics and then re-enters the reductive groundwater, DN become very active reducing the nitrates primarily to dinitrogen gas. The presence of DN in the well would be a warning that the well is being challenged, and possibly biofouled, by an upstream source of nitrates and organics. When a short time lapse is detected, it can be suspected that the biofouling in that well has at least in part been due to organics with a high nitrogenous content that had been exposed to near-surface oxidative conditions where the nitrates were generated.

Increases in the iron content in biomass, where cations are accumulating, follow commonly with iron released later in the maturation of the biomass. This iron is commonly in the form of ferric iron that initially will turn the color of the water to a yellow and later will cause particles of reddish-brown to appear. These may also collect on surfaces and be very obvious when the water is being filtered. For iron diagnosis, the bottom line is to recognize that the well is a natural filter and the upstream groundwater iron (mostly in the ferrous form) is likely to accumulate in the natural filters in, and around, the well in the ferric form. As this iron-rich groundwater moves through the biomass it may become accumulated into growths and so is taken out of the groundwater. This would mean that the groundwater pumped from the well would have a low, or virtually no, iron content (e.g., $<$0.1 ppm Fe). Iron being accumulated in the biomass causes major structural changes with the formation of encrustations. These are like a living porous concrete (bioconcretion) and can accumulate iron commonly up to 30% of the dried weight and sometime up to as much as 98% (to chemically resemble pig iron) with 70%–80% being a common upper limit for the iron content. During this bioaccumulation there appears to be

several stages in the iron saturation process which includes the shearing or sloughing off these iron-rich growths. Releases of iron from these growths appear to follow a sequence of events:

1. Yellow biocolloidal particles are released that turn the groundwater to a shade of yellow (yellow slime).
2. Red granular particles are released (red dust).
3. Iron-rich encrustations begin to collapse releasing large irregularly shaped particles that can easily get hung up on filters and surfaces. Iron content in the yellow slime averages about 8% but in the red dust it can be higher at up to 20%, while in the larger aggregates the iron can commonly be greater than 20%.

In the evaluation of water wells for possible biofouling with iron-accumulated plugging, one of the first symptoms should be the detection of iron (commonly in the ferric form) in the groundwater that has been pumped from the well. Given that the natural filters within the well are capable of naturally removing the iron from the upstream groundwater flowing into the wells environment, it would be expected that the pumped water should contain very low iron concentrations (e.g., <0.1 ppm Fe). Once the biomass that is accumulating the iron begins to fail then yellow slime is likely to appear in the downstream water. This would do two things to the water quality:

1. Change the color of the water to a light yellow possibly still with a high clarity.
2. Cause iron to be detected in the water at concentration commonly between 0.5 and 2.0 ppm.

Clearly for the operation of a well the appearance of iron in the pumped water when it had not previously been present would indicate that a problem is being generated. This symptom alone should trigger a preventative servicing of the affected well.

Increases in turbidity (cloudiness) occur when the biomass begins to enter an unstable phase due to a shearing/sloughing from the slime growths which then gives the water a clouded appearance. This is because the water is becoming loaded with biocolloids that can be detected as total suspended solids or more directly as particles that can be sized using the laser particle counting technique.

Some waters have a natural turbidity from development and so these wells have to be treated as having a different starting point to a well that delivers crystal clear water with no cloudiness. Cloudiness in water traditionally used to be thought of as originating chemically through the reaction between the chemicals in the water, particularly as they move from the more reductive upstream sources into the more oxidative downstream waters.

In the last 30 years, it has become evident that much of this cloudiness occurring in the water relates to the natural filters and the biomass that is positioned in, and

around, the wells. As this biomass grows perched in the formations around the well, it can start to become unstable. This causes some of the biofilms/slimes/biocolloids to detach and enter the groundwater stream being pumped from the well. If these detaching materials contain very little accumulates of cations such as iron then there would be very little color in the cloudiness. If there are cations being released (commonly dominated by iron) then the color of the water would tend to move towards yellow. If the conditions are fairly reductive and there is a significant concentration of sulfate in the water and SRB are active, then black (iron) sulfides would be formed to give the water a dark grey or black color. If there are no reactive cations such as iron to create these sulfides then the water may generate a strong "rotten egg" odor.

Either cloudiness or turbidity can be used to detect the early signs of biofouling in a water well that could then impact on the Q/s. Factors that could be considered significant in triggering a PM servicing treatment could be:

1. Increases in turbidity or cloudiness that is sustained through a 6-week period.
2. Sustained increases in the color of the water generally moving to a yellow (in the event of iron being in the biomass) to dark grey or even black if sulfides are being generated.
3. Generation of odors of "rotten egg" (hydrogen sulfide) being the most common although rotting vegetable odors are not uncommon.

Any of these three events could form an early warning signal that the well is in need of PM servicing before the problems get more serious.

Changes in the dominant bacterial types, as wells go through a plugging process causes microbes in the formations in, and around, the well to adapt and changes in the dominant groups. This shift in dominance created by the adaptation can also be used to mark that the well has a biomass that is adapting and changing. Such changes also can signal impending failures in the Q/s.

Another factor that can be linked to a water well beginning to significantly fail is a change in the type of bacteria that are detected in the pumped water from the well. During the early growth of the biomass in, and around, the well does undergo changes that can be associated with the natural pulsing of the biofilms (expansion, stabilization, and compression in a cyclic manner for 10–40 days per cycle until the biomass becomes too large and congested) such as changes in the reaction patterns observed for different BART testers. Typically, the shifts will relate to either the maturation of the biomass or the shifting in the redox front. These shifts are defined in Table E.2 with comments on the significance of these changes. Movements in the position of the redox front are likely to be very different for each of the three major types of water well. For vertical extraction wells, the most likely movement of the redox front would be towards the bore hole as the biomass grows. This means that there would be a greater likelihood of seeing the reaction patterns more typical of reductive conditions. Typically the biomass around these wells would extend 1–3 m (3–10 in.) out from the bore hole and would not have a truly cylindrical form but rather reflect the nature of the water flow into the bore hole with the biomass

TABLE E.2
Interpretation of BART Tester Reactions to Oxidation–Reduction Potential (ORP) Conditions in Water Wells

BART	RX	ORP Range	Comments
Iron-related bacteria			
IRB	CL	+50 to −50	First aerobic reaction followed by secondary Rx
IRB	FO	+10 to −150	First anaerobic reaction commonly followed by CL
IRB	BC	+50 to −20	Secondary aerobic in iron-rich biomass
IRB	BG	+10 to −100	Secondary or tertiary reaction in iron-rich biomass
IRB	BR	+50 to −20	Secondary reaction in iron biomass
IRB	GC	+50 to −20	Reaction when pseudomonads are dominant in IRB
IRB	RC	+10 to −150	Reaction when enteric bacteria are dominant in IRB
IRB	BL	0 to −150	Terminal reaction when enteric and pseudomonads active
Sulfate-reducing related bacteria			
SRB	BT	+50 to −20	SRB in aerobic consortium as part of the biomass
SRB	BB	0 to −150	SRB in anaerobic biomass often exclusive covert SRB
Heterotrophic aerobic bacteria			
HAB	UP	+50 to −50	Aerobic heterotrophs
HAB	DO	0 to −150	Anaerobic heterotrophs
Slime-forming bacteria			
SLYM	CL	+50 to −50	Common first aerobic and anaerobic reactions
SLYM	DS	+10 to −100	Anaerobic dense hydrogels (slimes) formed
SLYM	SR	+50 to −10	Aerobic dense hydrogels (slimes) formed
SLYM	TH	+50 to 0	Aerobic slime threads generated
SLYM	PB	+50 to −50	Pale blue fluorescence in higher organic flows
SLYM	BL	0 to −150	Terminal reaction when enteric and pseudomonads active
Denitrifying bacteria			
DN	FO	−10 to −150	Anaerobic bacteria reducing nitrates to dinitrogen

Note: The ORP range is in millivolts and is shown for each reaction (RX) as a common range for that particular reaction but it does not mean that an ORP shift has to occur between one reaction and the next. Other factors besides ORP may also dominate these changes.

maturing vertically at different rates depending upon the nature of these flows. In a well that has been designed to have a laminar flow, the biomass profile is claimed to form as an even vertical cylinder of growth. Vertical injection wells tend to have the redox front placed further back from the bore hole particularly when the injected water is oxidative (oxygen rich). If the injected water is oxidative then, over time, it could be expected that the biomass would creep closer to the bore hole. Sampling from a vertical injection well means either reversing the flow in the well in order to take the sample or trapping a sample at a downstream monitoring well. In either case, the water sample may be seriously compromised and may not reflect, in a realistic manner, the microbial activity within the biomass at the redox front.

Horizontal extraction wells tend to have very large slot area to length ratio compared with vertical wells (slot area to height ratio). This would mean that these wells would develop a redox front closer to the well. Due to the abundance of slots in the horizontal well, a condition can arise where the redox front and biomass are actually mobile, moving along the length of the horizontal well. Relief wells, taking the surplus hydrostatic pressure of an earth-filled dam, are subjected to biofouling focussed at two main sites. First site is the redox front that forms as the oxygen from the reservoir water is consumed during infiltration into the dam structure. The second site is as the water relieved from the dam now moves towards the relief well which again presents oxidative conditions. To rehabilitate relief wells, there are therefore two possible sites where the plugging biomass could have perched as the water moves down.

Information can be gathered by examining the reaction pattern signatures about the biologic activity occurring within the biomass fouling the well. Such data is likely to be of most value for extracting horizontal and vertical wells rather than injection wells. For injection wells, the best manner to determine the biologic composition of the biomass is to sample downstream from the injection point at a monitoring well. This could possibly create other problems associable with the monitoring well that is used to take the sample since these wells will, in all probability, have generated its own "natural filter" of biomass that could significantly affect the value of the data generated. It has to be remembered that a monitoring well designed to monitor basic hydrologic characteristics was not designed as a suitable method for the detection of "background" microbial communities in the groundwater. This is because there would inevitably be some formation of natural filters around the monitoring well that would bias this data in two ways:

1. Microbiologic content of the sample would reflect local biomass activities around the well that might not be representative of the active water wells that the well is being used as the monitoring device for.
2. Recovered chemicals in samples are likely to have been subjected to bioaccumulative and degradative processes within the entrenched biomass in, and around, the well that significantly impair the precision and interpretability of those samples.

While monitoring wells do serve a very significant purpose to determine movements in the water table and groundwater flows there is a significant

microbiologic challenge to their use as sampling sites for chemical and biologic parameters.

E.5 PM SERVICING TIMING

Once evidence has been generated (e.g., via Q/s, BART testing) that the well is beginning to fail due to biofouling, timing of a PM treatment should be based upon the state of the well and not the maintenance budget. For a newly developed well the PM treatments can be based upon the reaction scenarios already discussed above with the objective being to make the well sustainable. For an existing well that has not been monitored, but is now becoming sustainable, the first concern must be how much has the Q/s been lost and how active/mature is the biomass? Those concerns become a puzzle if there is not good data going back to when the well was first developed. In this latter event, this lack of a baseline means that the first PM should occur quickly in order to determine how badly fouled the well might be. For a newly developed well, the PM treatment is dependent upon any shifts in the Q/s, BART data, cloudiness, oxidation–reduction potential (ORP), or symptoms that create a concern for the sustainability of the well.

Water wells that are already operating and, possibly, have an inadequate data base, can immediately create a challenge since there is no legitimate base line to operate from. The logic approach here is to conduct a full set of tests as designated above and then perform a PM regardless of the data that has been gathered from that full set of tests. Six weeks after PM treatment is applied these tests are repeated to determine whether the PM had any impact on the Q/s and the activity of the bacteria being monitored. If there are no significant changes in the Q/s and other parameters after the treatment then this would indicate that the PM had been ineffectual. If the Q/s increased by more than 5% and the BART time lapses extended by at least 10% then it may be assumed that the PM treatment did have an effect. A second PM treatment should follow 6 weeks after the post-treatment testing and this should be followed by a full set of testing again. In the event that the second PM treatment showed similar increases to the first treatment then the well would appear to be recovering from the fouling. If both PM treatments had no effect on the characteristics of the well as monitored then that would mean the PM was ineffectual and a different strategy needs to be applied.

For an existing well that does not have a clear history of its performance characteristics it would be beneficial to apply a further regeneration treatment to that particular well using the pre-treatment data as the baseline. Recovery associated with the regeneration treatment would only be considered as moving the well back towards its original performance characteristics which are not known. In the event where the well has been newly developed, the first PM treatment would not be applied until after there were indications of deterioration in the functioning of the well as described above. This may mean that the well will not receive a treatment until 6 months or even as long as 2 or 3 years after the well went into production. As there is a better history for a well that has been monitored from new, it means that the PM treatment can be related much more accurately to the original characteristics

of the well. If the PM treatment fails to achieve any recovery then clearly the practice would need to be modified in order to ensure a satisfactory recovery. One feature on the operation of a PM treatment of a well is that it needs to be customized to meet the problems and challenges that are observed during the initial testing of the well.

E.6 REGENERATION TIMING

Regeneration of water wells is a major undertaking involving an adequate level of treatment to achieve the following:

1. Break up the biomass that is plugging the well.
2. Mobilize all of the fines that are clogging the well.
3. Open up pathways through which groundwater is moving towards the bore hole.
4. Remove any of the precipitates and debris that may have collected as deposits in the bore hole.
5. Clean off the biomass and associated materials from within the bore hole environment.
6. Remove all of the disrupted and dispersed materials from the well.

These are the six basic essential steps in conducting regeneration. There can be serious concerns related to the final disposal of the disrupted biomass and accumulated chemicals that have been recovered from the well. This final disposal becomes a critical concern where there has been a diverse range of cations including such elements (for example) as chromium, cobalt, arsenic, and boron that have accumulated in the biomass. Disposal would have to ensure that regulatory compliance conditions were addressed.

Regeneration treatment commonly faces two major challenges which are as follows:

1. Constructing a treatment program would give the best probability that the well under treatment will recover a significant amount if not all of the original quality and quantity characteristics.
2. Treatment would be undertaken by skilled practitioners who are experienced in the art and do recognize the needs to tailor the treatment to the fouling well rather than tailoring the well to their standard treatment protocol.

It is important that not only all of the data is relevant to the well examined before deciding on the regeneration treatment but also the practitioner "walks the well." This practice may seem futile since the well is under the grade and it is not (unfortunately) possible to see the plugging (i.e., biomass beast) and clogging around the bore hole that are the primary causes of the problem. Even camera video logging does not do this effectively since it concentrates on the bore hole itself with

just side long glances if the camera lens has the ability to be articulated and look sideways. Since the biomass beast will, in all probability, be skulking around out of sight, the problems may then be out of mind!

Walking the well also means talking to the operators, listening to their concerns, and taking note of their observations on the manner in which the well has performed in the past. This would include how the well was constructed, developed, and traditionally operated (continuously, periodically or used mainly as a back-up well). Even the manner in which the well is hooked into the pipelines for the distribution to the treatment plant and storage tanks may be significant since backflow into the well may be a significant concern. Another factor sometimes overlooked is where do the treatment chemicals (and heat) go when it is injected into a well? They will follow the line of least resistance that could mean bleeding away down stream of where it is needed through gaps in the plugging biomass. Horizontal wells also create problems because the nature of the redox front and the subsequent growth of biomass are likely to be laterally oriented rather than vertically oriented as would be the case in vertical wells.

In applying a regeneration treatment, which today commonly employs a blended treatment minimally with two chemicals, there is also the need to be sure that the treatment meets with local, regional, and national regulated standards. This is particularly important because, inevitably, some strong chemistry will be applied even if it is simply to break down the biomass and disrupt the clogging. Previous experiences with those types of wells in the same type of well field as the well to be treated now becomes very important to assure that the treatment becomes effective and economical. In doing this, it does not mean to say that the well will never need to be treated again. Sustainability means dedication, diligence, and durability in the testing, interpretation, and effectiveness of the regeneration treatment of the well.

It has to be remembered that effective regeneration means that the biomass accumulating inorganic and organic materials has been subjected to shock, disruption, and then dispersion. This means that these bioaccumulates will be appearing in high concentrations in the post-regeneration discharges. Very often the concentrations of some of the bioaccumulated materials is so high that the discharge can be considered a hazardous waste requiring diligent regulated disposal. While this groundwater is very hard with salt concentrations measuring in the range of 4%–8%, these levels of salt do not act as a deterrent to the growth of biomass and plugging. Disposal in this case is not limited to concerns for the high metal content in the discharges but also to the high concentrations of salt in the water that makes surface disposal impractical.

F Interaction between Neighboring Water Wells

There is a natural curiosity when water wells are close together and just how much they will interact with each other. Certainly there would be hydrological interactions particularly if the two wells overlap in their cones of depression (spheres of influence). This would have the following two major effects:

1. Direct competition for the local groundwater resource
2. Major changes in the microbiological activities resulting from these interactions.

While the hydrological aspects may be fairly easy to determine based upon the local characteristics of the formations and aquifer potential, the biological aspects become much more complex. This complexity can be the result of the two boreholes setting up competing natural filters that are influenced by demands created by each of the two wells for water. This would lead almost inevitably to greater movements in the water table, the vacillations of the redox front, and impact on the quality of the groundwater arriving at each of the two boreholes. These impacts would include competitive elements for the extraction of the groundwater that could have both negative and positive effects.

Negative impacts dominated by the increasing demands that would be placed on the aquifer within these locally affected regions caused by the two cones of depression as water is extracted from both wells. Biologically the interactions now become much more complex. It would be expected that the water levels within the impacted region would tend to have bigger vertical harmonic motions that would effect the movement of the redox front. Demands from two wells pumping at the same time would mean that the redox front would tend to be routinely drawn further down and closer in relation to these two active wells. There would therefore be an increasing potential for a larger zone in the formation that would be semi-saturated with groundwater over time and the biomass would be very active. Such events could increase the activities of molds (fungi) around the well in the lateral semi-saturated regions affected by the pumping demands of the wells. This might be reflected in sporadic higher counts of mold spores in the pumped water.

Perhaps the most significant potential negative aspect of two wells essentially competing for the groundwater would be found in the positioning of the natural

filters and associated biomass that would be generated. Given that redox fronts associated with both wells would not necessarily be in harmony, there would be a tendency for biomass formation to be generated over a larger region around both wells and have less dominance within the voids at the infected zones. This could cause greater variations in the microbial content of the pumped water due to the biomass being under increased stress that could lead to high levels of sloughing (sheering) during the disruptive stages of the life cycle impacted by the demands from pumping the wells. Additionally, this variation could lead to plugging events occurring over a larger region, which may initially exhibit less impact on Q/s for each of the neighboring wells with a greater probability of a more catastrophic collapse in production at some point in the future.

One of the positive effects of having neighboring wells pumping from within the same formation could be the results of shielding effect of the natural filters associated with the one well (upstream), which could to some extent protect the downstream well from polluted groundwater. For example, if there was a migrating plume of solvents moving toward the two wells, then the natural filters around the upstream well would act to bioaccumulate and degrade the solvents meaning that the plume would not so quickly impact the groundwater being extracted from the downstream well. Essentially the biomass (within the natural filters) of the upstream well would have acted to restrict the movement of the plume (through accumulation and degradation) so that the downstream well would remain free of the solvent for a longer period. Thus, the downstream well would not be so badly impacted initially by these solvents within the plume. Here, there is a significant impact on the upstream well because it would suffer from periodic appearances of the solvent in the extracted groundwater along with much higher populations of bacteria that would be associated particularly with the degradation functions under oxidative regimes. It may be argued that the upstream well is now performing a sacrificial role which allows the downstream well to continue to function effectively.

Interactions between neighboring wells is not easy to determine particularly when placed in an aquifer that is subjected to rapid movements of groundwater through fractures and, conversely, "perched" water that is being retained within water-filled lenses within the formation. Here, knowledge of the local hydrological conditions within the aquifer becomes a key factor in the determination of such impacts.

F.1 INTERACTION WITH NEIGHBORING OIL AND GAS WELLS

Water well owners quite naturally get alarmed at the sight of an oil or gas well being installed in relatively "close" proximity to their water well. There is quite naturally a concern that there will be an impact between the oil and gas well and the existing water well, even though the oil and gas wells are commonly much deeper in the geological formations than the water wells. Generally, oil and gas wells exploit formations that are measured in hundreds or thousands of

meters beneath the grade, while most potable water wells seldom exceed 200 or 300 m. This difference is because the water wells are extracting potable water that normally only exists in the upper aquifers; the oil and gas wells are more deeper set exploiting the oil and gas reserves set within groundwater formations that have a much high salinity. In regions where there are potable groundwater formations above oil and gas deposits, there is clearly a need to protect the potable groundwater when exploiting these deeper set hydrocarbon resources. Here, one major risk occurs during the development of an oil or gas well and it is important to ensure that it does not impact on the water wells set above the formation.

Drilling an oil or gas well often involves the use of surface waters as the principle source of fluids. This water is injected in the borehole as drilling fluid. To prevent significant leakage of these fluids into the aquifer for oil and gas wells, a short-term preventative approach normally employed is to inject loss control materials (LCM) to restrict fluid losses. Once the oil or gas well has been completed, various techniques commonly employing concrete are now put into place grout seals to separate the well bore from the outside environment. There remains a reduced potential biological risk to the aquifer as a result of the use of surface water in the drilling fluid and the use of LCM as temporary control methods to prevent significant drill fluid loss. For the users of the aquifer within which the oil or gas well is being installed, there is a natural concern relating to the biological integrity of the groundwater system becoming significantly compromised by these two events (surface water entry into the aquifer as drilling fluid, and the positioning of LCM to control flow from leakages where these occur). These concerns relate particularly to the period when the well is still being installed and developed and before permanent (leak) control structures are put into place. Generally, this period of time would extend commonly from 2 to 7 days in length.

Concerns relating to surface waters, in part, stem from the fact that they have very different biological characteristics to groundwaters. It can be expected that the injection of surface waters into groundwater can have some impacts, but these are likely to remain localized and short termed. There are a number of natural concerns when surface waters are used in this manner. Due to the very nature of groundwater (flowing through geological structures), the only organisms that would commonly survive and flourish would be microorganisms mainly because of their smaller size, greater ability to adapt, and less reliant upon oxygen or carbon dioxide for survival. Essentially the issue of microorganisms in the surface waters being injected into the groundwater as a part of drilling fluids can be separated into three areas of concern:

1. Health risk to the users of the groundwater impacted by the surface water.
2. Risks to the groundwater from microbes from the surface water adapting to and changing the nature of the impacted groundwater.
3. Potential for additional microbial growth in the groundwater because of the richer nutrient and oxygen loadings being carried at times by the surface water.

Health risk is a very natural concern for all users of any groundwater system that is being impacted by surface waters. Microbiologically influenced health risks in waters have been effectively monitored over the last century using coliform bacteria as the critical indicator group. These coliform bacteria occur abundantly in fecal matter and raw sewage and can therefore appear in surface waters that have not been adequately protected. Protection involves the physical removal of the solid wastes and disinfection of the liquids. Over the last 100 years, various forms of chlorine have been found to be the most effective disinfectants since the coliform bacteria are, in general, very sensitive to this halogen. Essentially the direct health risk from surface waters can be controlled by effective disinfection of those waters before they are used (primarily in drilling fluids). Even in the event that coliform bacteria do penetrate into the groundwater environment, conditions are generally not favorable for the long-term survival of these microorganisms. Commonly coliform populations will decline by as much as an order of magnitude each day. However, a few coliform bacteria (particularly in the total coliform group) are capable of adapting and flourishing in the groundwater environment. These bacteria can become integrated into the natural bacterial communities within the groundwater and do not normally pose a significant health threat to the users of that groundwater. It can therefore be considered that surface water, even if it possesses coliform bacteria, does not pose a long-term threat to the groundwater even in the immediate location of the new oil and gas well.

Injection of surface water into the groundwater would have some impacts. The following two major impacts are likely to occur immediately:

1. Higher oxygen content originating from the surface water.
2. Lower salt content in the injected surface water.

While the surface water would initially contain more oxygen than the receiving groundwater, it is highly probable that this oxygen would be consumed rapidly through reactive respiratory microbiological functions leading to the oxygen declining to background levels for the groundwater without causing any considerable biological impact. While the salt concentration in the surface water being injected is most likely to be lower than the groundwater, the effect of this difference is not likely to have a significant impact on the microorganisms within the groundwater environment.

Surface waters being injected into groundwater would be carrying a burden of oxygen and nutrients that could include living, traumatized, and dead cells; various forms of organic materials; and some nitrogenous and related compounds. In general, it would be expected that this input would elevate the natural nutrient levels in the receiving groundwater. The impact of this would be limited to:

1. Accelerating the rate of oxygen consumption.
2. Stimulating the biomass to become more active.

Here, the impact would be dependent upon the amount of microbial activity created. For these activities, there are two possible outcomes of significance. First,

this heightened microbial activity is likely to suppress the coliform bacteria more rapidly. Second, this greater level of activity could lead to a stimulated level of short-term plugging within the impacted site created by the installation of the well. However, over time there would be equilibration of the environmental conditions to those commonly associated with the groundwater. This would now cause any effects to be transient. Exceptions would occur if the surface water being used had an excessively high nutrient content or impacted using larger volumes into the recipient groundwater.

The other factor that could influence the biological activity around the oil and gas well as it is being installed is the application of LCM to control possible losses of drilling fluid into the formation groundwater. All LCM bear the following three common characteristics:

1. These materials are able to rapidly create plugging and significantly reduce drilling fluid losses.
2. All tend to be comprised of materials that would be resistant to rapid biological degradation (i.e., recalcitrant).
3. Have the ability to bind water.

These LCM are employed to achieve an effective control of fluid losses through binding water into colloids, plugging water flows, and slow rates of degradation. Applications are designed to maximize the efficiency of drilling and development of the well over a short period of time, commonly 1–5 days. Since rapid degradation would not allow the plugging and binding to be effective, all of the LCM use recalcitrant organics that do not degrade rapidly. This would mean that in the short term these LCM would not significantly biodegrade and so would not cause any major biological effects. The recalcitrant nature of these LCM means that subsequent degradation is likely to be slow and localized.

Surface water microorganisms tend to be more diverse in type than in groundwaters and occur at levels that vary with the season due to variations in temperatures and solar radiation. Such seasonal variations are not so evident in groundwaters particularly when they are below the influence of near-surface formations. Essentially these indigenous microorganisms can be categorized into three groups as follows:

1. Potential health risk microorganisms.
2. Microorganisms capable of adapting and growing in the groundwater environment.
3. Microorganisms that do not have an ability to remain active in groundwater situations and either enter some resting stage or are cannibalized by the other active microorganisms within the formation.

Each of these three groups has different implications when introduced by surface waters to groundwaters. Health risk microorganisms generate the most concern for users of an aquifer impacted by surface waters containing these microorganisms.

Of most significance are the coliform bacteria since these are accepted and regulated as the most significant indicator group for health and hygiene risks. In the public forum, it is the coliform bacteria that are considered the harbingers of health and hygiene issues (commonly focused on gastroenteric infections and to a lesser extent risks of becoming infected with pneumonia-type bacteria). Of the coliform bacteria, it is members of *Escherichia coli* that cause the greatest concern. Regulators in many jurisdictions have designated a zero-tolerance policy to total coliforms (which includes all types of coliform bacteria whether they are capable of becoming pathogenic in humans or not). Three factors shorten the survival potential for these bacteria. These are:

1. Coliform bacteria generally cannot compete effectively against the normal microorganisms present in the groundwater.
2. Many of the coliform bacteria including *E. coli* are not able to tolerate higher levels of salinity.
3. Chlorination is much more effective as a biocide against the coliform bacteria than against the indigenous bacteria naturally present in the groundwater.

Because of these observations, the presence of *E. coli* and/or other coliform bacteria are most likely to indicate that there has been a relatively recent introduction of coliform bacteria into that system.

Exceptions do occur for some of the total coliform bacteria that are able to compete effectively and become a part of the indigenous microflora in the groundwater. Bacteria able to do this commonly belong to the genera: *Enterobacter*, *Serratia*, *Citrobacter*, and *Klebsiella*. In Saskatchewan, Canada, for example, between 15 and 40% of the water wells in any given region are biofouling to some extent by these genera of total coliforms. This creates a challenge to the zero-tolerance policies that are in place for the protection of sources of potable water.

There are, within the groundwater, natural populations of indigenous microorganisms dominated by bacteria that live as stratified communities generally focused within specific ranges of oxidative or reductive environments with most of the biomass concentrated at the redox front (here the environment moves from a reductive to an oxidative state). These microorganisms are capable of adapting and competing with the natural microflora in the groundwater and so will end up being assimilated into the melting pot that is the natural community. This group of microorganisms is dependent, for their activity and survival, on the amount of nutrients arriving with the drilling fluids as well as their ability to adapt and compete effectively against the incumbent microflora. This would challenge the incoming microorganisms to not only acclimatize to the local conditions quickly but also compete effectively against established microbial communities. These natural communities would, for the most part, have already attached to the most suitable surfaces or have been incorporated into preexisting biocolloids (plugs, slimes, and floaters). Within the communal "melting pot" of the groundwater, it is most likely that adaptable microorganisms would not achieve any dominant hierarchal roles unless environmental conditions were changed dramatically by the admission of the

surface waters as a part of the drilling fluids. This would then be a "local" impact rather than a regional one.

Another group of microorganisms go into some form of dormancy within the groundwater. This could happen where the microorganisms are unable to adapt to the groundwater environments. This would mean that the cells that could not adapt would likely enter a state of trauma culminating in either the formation of spores, entering a dormant phase or dieing out (through the formation of spores or suspended animation cells, ultramicrobacteria) or eventual natural disruption of the cells with the contents becoming a part of the nutrient train for the indigenous microflora.

In summary, surface water differs from groundwater primarily in carrying a higher concentration of oxygen, less salts, and occasionally a greater biological burden. All of these differences can have an influence upon the receiving groundwater for such surface waters. It needs, however, to be recognized that the receiving groundwaters commonly would have a much greater total volume than the applied surface water. While this dilution effect is very significant, due diligence is however necessary to ensure that the surface waters do not have significant impacts upon the groundwater particularly when applied in a drilling fluid. While the dilution factor of surface water to groundwater is in itself a mitigating factor, there are a number of significant considerations that could result in a sequence of impacts on the groundwater environment. Clearly, these impacts can be controlled to some extent by the appropriate application of drilling fluid additives as well as any LCM to contain these effects. Impacts are categorized into four probable sequences within which they can be expected to occur.

F.2 OIL AND GAS WELLS

F.2.1 Impact One: Oxygen and Dilution Impact

Surface waters will normally carry some dissolved oxygen down into the groundwater where the concentration would be diluted by the groundwater (which would have lower or no oxygen in it). This oxygen would be utilized by the indigenous microorganisms in the water for respiratory functions. This creates a more oxidative zone in the injected region could now cause the generation of oxidized metallic cations such as ferric iron to separate out. However, these events would be of relatively short duration of this impact because of the large volumes of the surrounding groundwater.

F.2.2 Impact Two: Water-Borne Pathogenic Microorganisms

Surface waters may contain water-borne pathogens. These are most commonly detected indirectly using the presence or absence of coliform bacteria as indicators of health risk. As an indicator group, the coliform bacteria have proved to be effective for assuring the hygienic safety of water. However, these bacteria are impacted in a variety of ways by the receiving groundwaters. These impacts extend from:

1. Relatively high sensitivity of coliform bacteria to chlorine compared with other microorganisms
2. Vulnerability of coliform bacteria to competition from the indigenous bacteria in the groundwater that could suppress or eliminate the coliforms
3. Incorporation of some of the total coliform bacteria into the growing biomass.

Essentially the coliform bacterial numbers in the drilling fluids are most likely to become compromised by these various interactions with the coliform bacterial populations tending to fall rapidly.

F.2.3 Impact Three: Biofouling Potential

In drilling fluids impacting with the aquifer groundwater, there is also a potential for the microorganisms present within the zone of effects to respond by refocusing their activities as a response to the surface waters and other materials entering the groundwater. These interactions could involve a shifting in the biomass responding to nutrients, the changing oxidation–reduction potential, and/or application of surface areas that would be presented by the solid materials forming a part of the drilling fluids. Nutrient impacts are initially likely to be stimulatory to the biomass but generally short lived. It would be expected that the groundwater would shift to more reductive conditions where less complete degradative functions would then likely dominate. With this event happening, it may be possible for microbiologically influenced biofouling to become dominated with the generation of gases (e.g., methane and hydrogen sulfide) and acids (dominated by fatty acids under reductive conditions).

If there is a significant component of ammonium–nitrogen in the drilling fluids, then nitrates are likely to be end products under oxidative conditions if oxygen is present in the fluids. If there is a high sulfide content in the drilling fluids or formation materials in the aquifer, then there could be a potential for acid production by the activities of the *Thiobacillus* bacteria. In some parts of the world, acid sulfur soils are a major economic and regulatory concern, but in the prairies there is usually adequate buffering capacity to avoid these problems.

F.2.4 General Discussion

While biological forms and associated activities have been well documented in surface waters, the nature of biological activities in groundwater is not so well understood. Biological activity in surface waters is very much affected by the seasons and nutrient loadings. These cause periodic blooms in the levels of microbiological growth. In the aquifer environment, the groundwater becomes less and less influenced by the seasons with depth. As the depth increases, the groundwater tends to slowly get more brackish and then saline and conditions become more reductive. With the increases in depth, it is also commonly considered that the dynamics of the biological activity slows down. Insertion of surface water

into an aquifer therefore will have some potentially stimulating impact on the biological activity stemming primarily on the introduction of oxidative conditions with a greater organic load. Some of these impacts will be ameliorated by the materials applied to make the drilling fluids. Some factors controlling the biological impact are summarized in Table F.1. While these impacts are commonly recognized, there will be variations resulting from the precise form of the LCM and/or drilling fluids that have been used as well as differences in local environments within the impacted aquifer and the source of the surface water employed in the constitution of these materials.

For the operator of a water well that is suspected to have been impacted by the installation of oil or gas wells in the immediate neighborhood, there are a number of factors that could affect this impact as follows:

TABLE F.1
Probability of Biological Impacts Associable with Injected Surface Waters into Gas Wells During Construction

Factor	Immediate	Short Term	Long Term
Oxygen	Stress for anaerobic bacteria	Oxygen utilized for respiration by indigenous bacteria	Oxygen now absent unless the organic loading is so low that there is a little demand for oxygen
Escherichia coli	No effect on *E. coli* population	Gradually declines in *E. coli* population	Eliminated
Total coliforms	No effect on population	Majority of total coliform bacteria die off while minority adapt and grow in the ground water if conditions are suitable	Small minority of total coliforms introduced in surface water integrate into the bacterial flora in the groundwater
Ammonium	No changes in concentration	In the presence of oxygen, there may be some nitrification by bacteria to nitrates	In the presence of oxygen there may be complete nitrification by bacteria to nitrates
Nitrate	No changes in concentration	In the absence of oxygen and the presence of organics, nitrate will be denitrified by bacteria to nitrogen	Under reductive conditions, nitrate will be eliminated
Chlorine[a]	Residual chlorine will impact on some of the living microbial cells	Residual chlorine neutralized by the biomass present in the groundwater	None unless very high concentrations of chlorine have been applied

Immediate, activities that would occur in less than 2 min; short term, the time period from 3 min to 1 month; long term, effects that would not be observable until after 1 month or longer.

[a] Those forms of chlorine that commonly applied as disinfectants.

1. Both the water well and the oil or gas well are functioning at different depths commonly with the geological formations in between that can act as barriers to the free movement of the oil or gas.
2. Installation of oil and gas wells uses a common practice to ensure that there is a minimum of impact during the drilling process. In the event that a flow loss is noted during this stage of the drilling, the operator would use LCM to plug up the movement of fluids out from the well.
3. Once the well has been installed, the length of the casing down to the formation, from which oil or gas is being recovered, is commonly grouted using cement.

Upon completion, the new oil and gas well should have been sealed from the potential leakage of oil and gas into the upper formations and into the adjacent potable groundwater systems above. One major problem is that there are natural leakages of oil (particularly volatile petroleum hydrocarbons) and gases (particularly methane) that can occur and move up into potable groundwater supplies. This natural bleeding of hydrocarbons (including methane) can lead to elevated concentrations of organics (primarily from the oil and gas) in the overbearing groundwater systems. Since the conditions are reductive, these materials will not degrade (although they could be bioaccumulated) until oxidative conditions occur. This is most likely to happen at the normally lateral redox fronts present in the overbearing soils and water wells. In the latter case, the biomass within the natural filters in and around the water well would serve as the primary site of bioaccumulation and then degradation. Releases of these organics into the well water are most likely to only occur when this biomass has become saturated. Such events may take many months if not years to allow these gas or oil products to become determinable in the pumping well waters from an impacted well.

F.3 INFLUENCE OF MICROBIAL ACTIVITIES ON FUNCTIONING OF TILE DRAINS

Tile drains and leachate collection systems can be vulnerable to biofouling. Here, the water may have percolated through some porous materials before being collected within some form of perforated pipe collection system. Tile drains generally control the water table in the soil to prevent water damage to property. In leachate collection systems, perforated pipes are employed to collect leachate formed at such sites as sanitary land fill operations. In both cases, water pours through a porous medium that is sometimes highly reductive and, in other cases, oxidative. Both of these types of drainage systems commonly employ a sloping drain pipe that carries water or leachate away for disposal. These drain pipes are positioned to allow water to move into the pipe through perforation or slots and are sloped to allow effective drainage away from the site of concern toward disposal or treatment. Once the water or leachate enters the sloped drainage pipe, there is a greater probability that air will be entrapped within the environment. This would mean a more oxidative regime would now be generated if air is present where biofouling could become more aggressive.

For many leachate systems, the draining fluids will still remain reductive but moving toward the redox front-type conditions. Common differences between a tile drain and a leachate collection system is that the tile drains are generally oxidative with a low throughput of organics, while the leachate collection system tends to be reductive with high throughputs of organics. Similarities relate to the fact that both systems utilize a technique to divert and drain water from a saturated porous medium to the pipe which generally has a downslope to allow drainage. These systems are also vulnerable to biofouling but in different manners that are discussed below.

Tile drainage systems generally operate at shallow depths, commonly less than 3 m and have the function of relieving hydraulic pressures from engineered structures during periods of high rainfall, flood, or snow melt. These drainage systems are set into trenches that normally use a slight slope in the collecting pipes to drain water away from the site. Pipes normally have some form of perforations that would allow water to enter the pipes through the sides and are set into the trench and backfilled into place. On some occasions, the pipes are fitted with socks (a natural or synthetic material that is either woven or perforated). These socks are designed to act as a filter and prevent the entry of fines, plant roots, and other coarse material into the body of the pipe, but allow the water to pass through. Modest slopes to the socked perforated pipes are designed to carry the water in the formation away to a suitable drainage site such as a creek or ditch. This sock–perforation interface can then become a focal site for a redox front that may therefore support redox-focused biomass leading to a plugging of the perforations and failure in the drains.

In the event that a socked perforated pipe is used to collect, move, and finally discharge the waters that had perched over the drains, there are a number of potential biofouling challenges that could develop. These sites for potential biofouling may be summarized as follows:

1. In the fill material behind the outside of the sock.
2. In the permeable sock material itself.
3. Within the gaps in the pipe where perforations have been applied.
4. Within any stagnant or flowing water within the pipe.
5. On the walls of the pipe.
6. Where air cavity may be present in the pipe when it is not saturated with water limiting water flows.
7. At the point where the water is discharging away from the tile drainage system.

The source for nutrients to stimulate the biofouling would be carried by the upstream water moving into the tile drain through the backfill material that is surrounding the drain(s). Given that the functionality of a tile drain is commonly to remove any threatening, hydraulic head would build up, and then it would normally be expected that, in a functioning tile drain system, there would commonly be an environment within, and above, that was only semi-saturated with water while the environment below would likely be saturated. This would mean under those

circumstances that there is a high probability of air being present within the tile drains. If air is present, then the water inside the tile drain would most likely be oxidative because of the available oxygen. It would therefore be a reasonable probability that any biofouling would be aerobic with the greater generation of biomass than that would have occurred under reductive conditions. A greater biomass would be generated by the more complete utilization of any organics that occurs under oxidative conditions through respiration.

Since draining water has been collected primarily through the perforations, any organics, other nutrients, and chemicals being carried by the water could have also tracked into the tile drain. Most inputs that could be carried by the water through the perforations into the tile drain would come from either water pooling above the tile drain and nutrients being extracted from the formation materials around the tile drains (e.g., sands, silts, and clays in the backfill). A focused redox front would be created at each perforation through which this upstream water is moving down into the drain could be a site for plugging. Here, there would be a gradient created between the oxidative conditions within the draining tile drain and the more reductive outside conditions in the water-saturated fill material. This redox front position now becomes critical to the nature of any biofouling in or around the perforation. If oxygen is able to permeate beyond the perforation into the formation, then the biomass may focus upstream in the fill material causing hydraulic compromises as the permeability of these fills can also be compromised by the plugging events.

In the event that a sock has been used on the outside of the perforations, there is a greater probability that some biomass will aggregate within the entry points built into the sock causing rapid losses in transmissivity (i.e., causing plugging). Effectively, the entry points (pores) through the sock would have become plugged with biomass (slime, etc.) utilizing oxygen present in the headspace air and water, and the nutrients traveling toward the perforation in the water. Growth could therefore occur both outside of the perforation where upstream water is moving though the fill and picking up nutrients and also within the sock itself. Such growths would severely limit hydraulic transmissivity through the biofouled perforation but, if only a few perforations were impacted in this manner, then there would still be no noticeable effect on the performance of the tile drain. However, if there was a significant impact on the majority of the perforations, then this would be observed through the water perching above the tile drain and through not being able to drain through efficiently. This could then compromise the engineered objectives designed into the tile drains. Geotextiles are designed to provide a filtration function for removing fines from water. Normally these textiles are interposed with perforations through which the water subsequently flows. A weakness in the geotextiles is that they can also site for the growth of biomass that would reduce or totally prevent the passive movements of water. In landfill drains these geotextiles when positioned behind perforations can cause a combination of black slime and crystalline build ups that totally plugs (prevents) water flow. In a landfill operation the drains were totally plugged up but were recovered by the application of Blended Chemical Heat Treatment regeneration. Another example was the use of geotextiles on a slope to prevent surface erosion and the textile was pinned down with rocks. Here there was

so much gas formation under the plugging geotextile that the textile developed gas pockets between the rocks. A swift puncturing of the textile caused the gas to escape and caused the textile to deflate. Wrapping geotextiles around monitoring wells to cover the slots or perforations has two negative effects:

1. The biomass growing in the geotextile impairs the movement of water into the well.
2. The biomass acts as a natural filter removing many of the chemicals from the ground water before it enters the monitoring well. Data from such wells may therefore be compromised in two ways by the biofouling geotextile to cause a loss of precision to the hydraulic characteristics being generated and unstable chemistry that may not relate to the characterization of the regional groundwater.

Water that does enter into the tile drain through the perforations now enters generally a much more oxidative environment particularly if there is any headspace oxygen present in the air. If headspace air is present, then the water would be expected to flow down the walls (for those perforations set above the water level) or diffuse into the water directly (if the perforations are set below the water level). Biomass is most likely to be generated on the walls of the tile drain and within the water moving through the tile drain. Video camera logging can reveal these biological activities occurring within tile drains. This is commonly considered to be either plant roots that have penetrated the perforations or iron ocher that has been formed chemically through the dissolved ferrous iron (in the upstream water) becoming oxidized to insoluble forms (ferric oxide and hydroxide). In the event that a sock has been applied to the outside of the tile drain, it cannot be expected that plant roots could easily penetrate unless the sock material was compromised. However, some slime growths can resemble plant roots and the ocher usually observed is actually a product of microbiological activities.

To clean these types of sock textiles is particularly challenging since the biomass would have penetrated through the geotextile (if used) and out into the fill material. Physical disruption by jetting accompanied by chemical disruption using a combination of pH adjustments and penetrants would be essential to break apart (disrupt) and disperse the biomass. It should be recognized that this type of treatment would not be as effective as a disruption/dispersion agent in the fill formation and there would be a potential for fines released by the treatment to move closer to the textile.

This biofouling (primarily plugging) within the tile drain may not always represent a serious impediment to the flow of draining water through the passage. It would become more significant when the plugging of the slots and/or perforations now restrict the flow of water into the tile drain. In extreme cases, a video camera log of the drain may show the interior to be open and free from significant biofouling but the slots and/or perforations may be discolored and dripping with slimes or encrustations. These symptoms would signal that the tile drain is effectively plugged and water levels would now be perching above the drain increasing the risk of flooding.

Tile drains would not, at the first level of management, be viewed as having high biofouling risks but could reduce the transmissivity of water (drainability from the tile drain). In reality, risks do exist from biofouling particularly when it begins to plug up particularly at the access points (slots or perforations) leading to a failure in the weeping tiles or drains.

G Standard Microbiological Approaches for Groundwater Challenges, Concepts (Summary)

Appendix G has not been written to simply duplicate the many standard methods that are well established and are in confidant common usages in certified microbiological laboratories within the industry. This is more to define the protocols and methods relating more specifically to the role that microorganisms do play in the water industry (particularly in connection with corrosion, plugging, and health risks). At this time (2007 CE). There is a heightening public neurosis with the risks that microorganisms present to human health and a diminishing understanding of some of the "good" roles that many microbes play.

One effect of this preoccupation with the risks (of catching some infection or other) is a plethora of anti-bacterial soaps, creams, and chemicals that are implicitly claimed to control the risk but very few really do! Another effect in the general public of this avoidance of public water supplies (even though community supplies are well regulated) in favor of various forms of bottled water that are considered safer even though not so heavily regulated.

In these standard methods used in microbiological determinations of microbiological populations and activities (including risk) the emphasis is placed on:

1. Obtaining an acceptable water sample for the testing (Appendices H, I, J, and L).
2. Conservation of the water sample between collection and testing (Appendices H and I).
3. Defining the influence that ambient water temperature may have on the confidence in the microbiological test data based on specific incubation temperatures (Appendix J).
4. Obtaining water samples that do reflect the activities associated with attached (often encrusted) microbial growths (Appendix I).
5. Application of the BART testers to determine the nature of microbiological (community or consortial) challenges within a given groundwater system (Appendix L).

6. Functional advantages and concerns of molecular and genetically based determinations of microbial presence and activities (Appendix L).
7. Risk determinations using analytical methods relating to specific capacity, plugging, corrosion, and health risk events and treatment evaluation (Appendix M).
8. Sustainability in water wells (Appendix M).
9. Regulatory considerations (Appendix N for phosphorus only).

There can, therefore, be no question that many bog iron-ores owe their formation largely due to the activities of iron-bacteria. Molisch's results are interesting because they confirm what could be conjectured to be the case from an examination of the ochre-beds of the present day ferruginous waters. Ellis, D., 1919, *Iron Bacteria*, London, Methuen & Co. Ltd., 167.

H Methods for Obtaining Water Samples for Microbiological Testing

Something that is sometimes forgotten in the development of data using a variety of analytical techniques (that may have cost a thousand dollars or more) is that this collected data relates only to that sample as collected and handled prior to the analysis. As such the data may not provide relevant information for the fuller evaluation of the events that may be occurring in the system from which that water sample was collected, preserved, and analyzed. There are many stories of water samples being taken from dead-ended lines without any flushing (water after all is just water to many!) and generating horror stories due to the high levels of metals (including iron) and organics that have bioaccumulated within such lines. Sampling ports that have not been properly disinfected (sterilization being not attainable with ease at some sites) would also "bleed" microorganisms during particularly the first flush of sampleable water. If the site within the water system to be tested is back beyond the sampling port then another challenge would be to get water from that site of interest without it being diluted with other waters from different sites along the way.

Water wells are particularly a challenge because not only is there the need to sample from a pumpable borehole but also from the surrounding biomass that is likely to have clustered as a series of concentric cylinders of growth. Each of these communities would have different microbial dominance and characteristics. Clearly in a case like that no single sample is going to be able to represent all of the different microbial groups that could be associated with that biofouled well.

In the decision to take a water sample, clearly there is a need to consider exactly what the objective would be. From the microbiological perspective, the water entering the sampling port for collection has passed over whatever attached biomass (in such forms as slimes, encrustations, nodules, and tubercles), and has also moved through water containing suspended particles (biocolloids) that could contain active microorganisms. There is therefore only a remote possibility that a water sample then would be totally from the site that is desired to sample. The challenges relate to the dilution and diffusion of the sample with neighboring waters during the sampling procedure. There remains a significant possibility that the water will be compromised to some extent by suspended microorganisms in the water. If it is desired to determine the nature of the attached biomass (given that more than 90% of

the microbes are likely to be in this form) then some means of detaching at least a part of this biomass from the surfaces becomes essential to allow the testing to proceed with at least some level of precision.

One general rule that governs these events is that the attached biomass develops a harmony with the demands that are caused by the water flowing over the biomass. Even if the flows only occur for a few minutes every day this would still develop a stable biomass because of the constancy in the demands created in the well. To cause detachment of these biomass growths, it is essential to radically change the local environment from which the sample will be taken. For a managed flowing well then shutting down the well ideally for 7 days would cause the redox front to move towards the borehole triggering trauma in the attached biomass that had been growing at the front. This would mean that these detached microbes would now be in the water sample and detectable.

The voids within the fractures and porous media would also become loaded with microbes that have become traumatized and moved into the water. Sampling this water would therefore give a better appreciation of the microbial communities within this attached biomass and the environment from which the sample was taken. Many would not want to shut down water wells for a week simply to get a better understanding of the attached biomass causing plugging, corrosion, and health risk problems. Effective minimal times for a shut down prior to sampling a producing well should be overnight or 8 h so that at least some of the attached microorganisms would move into the water and be recoverable in the sample. Less than 8 h of shut down (e.g., 4 h) has been used where the well operation dictates that a longer shut down would not be practicable. When this is done the data loses some of its precision since the shut down may not have been long enough to cause significant movement from the attached biomass into the ground water.

Sampling from a well that is not normally being pumped, or is used as an injection well, would require that that well be pumped as an extraction well in order to cause the necessary trauma to release some of the microbes from the attached, entrenched biomass into the water. Such pumping should be conducted minimally for a period of 24 h in order to pull the redox front towards the well and traumatize the microbial biomass. Practical requirements may however not allow this but 4 h would then form a practical minimum.

Sequential sampling during pumping can be used to develop an understanding of where the (now traumatized and suspended) microbes were growing in the biomass in, and around, the borehole. When there is a sequence of sampling of the pumped water stream, the microbial contents of that stream will change to reflect the microbial composition from that location within, and around, the borehole. Early samples into pumping would reflect the microbial communities closer to the well while later samples would reflect microbes that were detached from further away from the borehole. This technique involves a shut down to cause trauma in the microbial biomass leading to the movement of microbes into the water, followed by a sequential pumped sampling and testing at specific times during a continuous pumping of the well. BART-SOFT™ (version 5.0) includes the ability to determine the positions of the various microbial communities using the zones of interrogation projections (ZIP) function that can then display the communities as concentric

circles around the well. Activity levels are displayed for each community so that the position and extent of each microbial community can be established. Undertaking a ZIP can become a key factor in the establishment of a regeneration treatment that would return the water well closer to its pristine condition.

I Conservation of the Water Sample between Collection and Testing

Water samples, once collected, become subject to a combination of biological and chemical activities that could affect the precision of any testing applied to that sample. Ideally, all water samples should be tested immediately using all selected procedures right at the site of collection. This is not likely to happen for all testing given our present ability for testing at site. There is always some period in time between when the sample is taken and the tests are performed which may be considered to be the sample storage time. For most chemical testing, inhibitors can be added to stop biological activity so that this does not interfere with the accuracy of the chemical test. At this time there are no chemical inhibitors that can reliably stop all biological activity in its tracks but still allow that activity or biomass to later be measured in a reliable manner. Sample storage then becomes one of the most critical issues in microbiological testing since the ideal would be to see no changes in the activities, or populations of microbial cells from the time of collection to the time of testing.

Even if a water sample has been taken with all due diligence for microbiological testing, there is the challenge of those changes that will occur in that sample from the moment it was collected to the moment that the testing starts. Given that the sample is for biological testing, it is not possible to simply add an inhibitor to stop all biological activity. Such inhibitors are most likely to be differentially toxic and thereby change the nature of the microbial community. Ideally, the shorter the time between collection and the start of the testing, the lesser will be the effect of the sample storage (time between collection and testing) on the validity (precision) of the microbiological tests. If the water sample contains chlorine that could impact on the survivability of the microbes in the sample then sodium thiosulfate tablets can be sued to neutralize the chlorine. In the BART testers, sodium thiosulfate is automatically included as a part of the selective medium to minimize the risk of the chlorine inhibiting the microbes.

Another major problem associable with sample storage is the potential for the temperature and the oxidation–reduction potential (ORP) conditions to become modified and shift the microbial activities occurring within the sample. Storage temperature is another major concern since there is a risk that, if the temperature moves outside of the original ambient temperature range for the collected sample, then there could be sufficient shock generated to change the activity levels of the

microbial cells. Some cells could go into trauma (effectively becoming suspended animates and technically inactive or dead).

In water wells, there is commonly a concentration of the active biomass around the redox front. In taking a water sample, particularly given the abundance of oxygen in the air, there is a strong possibility that the ORP value in the sample will shift towards an oxidative state at the same time as the redox front collapses during sampling and collection. It should be remembered that valid ORP tests can only be performed effectively on newly acquired mid-stream samples. Oxidation–reduction potential readings taken on stored and shipped samples are most likely to be meaningless because of the impact of air (or lack of air) during sample exposure and agitation during shipment along with the interaction with any headspace gases. Validity for ORP testing is most important when determining the effectiveness of a well treatment. Here, samples taken from continuously pumping sources may reveal the ORP gradient as the water comes in from further away from the borehole. Such testing across the ORP (redox) front would automatically be determined by the changes in the values obtained during the testing (going from positive to negative millivolts).

Water samples designated to microbiological testing need to follow a number of "rules" to ensure that the test remains sensitive and repeatable. These rules include:

1. Conduct the test as quickly as possible after the sample has been taken.
2. If the testing can be performed within 4 h of the sample being taken then the water should be kept at as close to the original ambient temperature of the water sample ($\pm 5°C$).
3. When the testing is delayed for more than 4 h after collection then the water should be stored over ice to suppress microbial activity.
4. In the event that the water sample is going to be kept for longer than 24 h prior to testing then the sample should be kept over ice with the understanding that the precision of the test will suffer the longer the sample is stored.
5. It should also be noted that samples kept in closed containers (with no access to oxygen) are likely to become gradually more reductive as the respiratory activities based primarily on oxygen and secondarily on nitrate, nitrite, and sulfate, become suppressed.

Even certified laboratories sometimes have difficulty in generating comparable data (such as by performing round robins) on a given sample from one laboratory to another simply because of the manner in which the original composite sample behaves during transportation. Unlike chemical testing where duplicate samples usually will show reasonable correlations, the complex additional factors associated with the living microorganisms can seriously affect reliability. One consequence of this problem is that microbiological test results are sometimes viewed as being of lower significance because of this additional uncertainty (seen as a lack of precision) when compared with chemical testing. Here chemical testing often involves parameters which are not affected by biological action (such as total phosphorus, calcium, sodium, magnesium, and potassium) and so tend to be relatively easy to determine with precision.

J Influence of Ambient Water Temperatures on Tests

Water temperatures from which samples are taken can have an impact on the precision in the microbiological cultural test data based on the specific predetermined incubation temperatures used. One factor that has to be considered in biological testing is the incubation temperature since microorganisms living in, or around, water wells tend to have very stable temperature conditions (unlike soils and surface waters which are subjected to day–night temperature and seasonal fluctuations). In the last one and a half centuries, microbiology has been dominated by the search for microbial pathogens of warm blooded animals (PWBA) that commonly function over temperatures ranging from 35 to 45°C. One unfortunate outcome of this was the idea that all microorganisms would inevitably grow best at these temperatures (35°C–45°C). By the 1930s it was realized that many microorganisms have lower growth temperatures that were optimal (most favorable) and by the 1970s, 28°C–30°C was considered to be the most suitable for these microorganisms. Today many tests employ this temperature range (28°C–30°C) for cultural testing of non-PWBA. Today, there are four temperature ranges that can be considered for the detection of microorganisms by cultural testing. These are: 12 ± 2°C; 22 ± 2°C; 28 ± 1°C; and 36 ± 1°C. Each of these temperatures is used for different communities of microorganisms that can be present in groundwater environments.

Lower temperatures are common in water at depths of 300 m (1000 ft) or more in the temperate regions and very often these wells have groundwater temperatures that are below 15°C for the greater part of the year. Natural incubation temperatures to culture these microorganisms can therefore be most effective at 12 ± 2°C. Here there would not be severe traumas amongst these cold-loving fractions of microbes that could occur when the temperature gets up to greater than 16°C. Many of the microorganisms growing under these cold-loving conditions can also grow at higher temperatures and these can be cultured at 22 ± 2°C (room temperature). Microorganisms cultured at this (room) temperature would not be growing at their moist favorable rate (optimal) but there would be a broader spectrum of microorganisms able to grow including many of the cold-loving and some of the warm-loving microbes. This temperature range (22 ± 2°C) has the convenience of being normal room temperatures in most countries and so becomes easy to set up.

That is one of the main reasons why the original BART testing was recommended to be done at room temperature. Care should however be taken to ensure that the temperature does not fall below 20°C since the range from 16 to 19°C can produce erratic cultural activities and a lower precision in the data generated. For microorganisms growing in the temperate environment where temperatures range from 16 to 34°C, the optimal incubation temperature has been found to be 28 ± 1°C. Using incubation temperatures higher than 29°C or lower than 27°C there tend to be a loss in precision. For warm groundwaters in tropical climates and mildly geothermal extraction wells, the microorganisms would operate over ranges similar to the PWBA and the most suitable temperature for culturing these microbes would be 36 ± 1°C.

Population counts (achieved by agar spreadplates as colony forming units, cfu/mL), or predicted active cells (achieved by BART testing systems generating time lapses convertible to predicted active cells, p.a.c./mL) are both responsive to the incubation temperature in different ways. For the agar spreadplate, the lower incubation temperatures do significantly affect the length of the incubation time to generate colonial growths. Generally the times before counting colonies are (temperature range followed by time length in days for testing): 12 ± 2°C, 21 days; 22 ± 2°C, 10 days; 28 ± 1°C, 7 days; and 36 ± 1°C, 5 days. For colonies to be countable, they first of all have to be able to form a large enough size to be countable (minimum, 0.2 mm, average diameter, > 2 mm) with the agar is provided with enough water and nutrients to allow growth. Colony counts will generally underestimate the number of microorganisms because of two factors:

1. Only a small fraction of the microorganisms are able to grow under the conditions that are generated by the agar medium.
2. Competition between rival colonies as they form will mean that some colonies will be impacted before becoming large enough to be countable and so will not be counted.

As an alternate to the agar spreadplate, it is possible to use the BART tester approach where the microorganisms in the sample are presented with a variety of environments within the diffusing selective cultural medium that are changing as the incubation continues. Active microorganisms are able to select and grow in the most favorable environment within the BART tester are likely to be detected by distinct reaction patterns after a recognized time lapse. Activity means that the cells are functioning and capable of growing quickly under the selected conditions.

K Protocols to Obtain Water Samples

It is very important that the sampling protocols reflect the activities associated with attached (often encrusted) microbial growths which may not be easily found in the water sample. Groundwater can emerge from an extraction well with very low turbidity (cloudiness), have a high clarity, and be free from significant microbial populations. Such water does not indicate that the well is free from microbial infestations and is not biofouled. It means that the sample had not picked up, or been impacted by, the microorganisms growing around the well. In other words, appearances can be deceiving! In practice, there is no such thing as sterile water in a well and so the question becomes whether the microbes are growing suspended in the water, or attached to surfaces? The challenge is often that $>90\%$ of the microorganisms infesting water wells are attached to surfaces in the biomass while only the remaining $<10\%$ occur floating in the water mainly in suspended biocolloids.

Video-camera logging of a borehole will sometimes show these floating microbial biocolloids as either clouds that slowly swirl slowly through the water column or as "jelly puddings" with suspended materials that appear to be glued or gelled within the water. As the camera descends through such slime structures the suspended materials will maintain their position until the compressive effects of the waves created by the camera moving cause the structures to collapse. At the same time, the camera may also scrape off attached microbial growths from the walls of the borehole. If these collisions occur then larger dense particles can be seen tumbling downwards while the lighter particles obstruct the field of view. When approaching the bottom of the water column there is also likely to be a disturbance of the bio-precipitates that have collected inside the base of the well below the screen. Disturbing this densely packed material can cause the camera view to become obscured until the camera is lifted steadily upwards and out of the disturbed zone. All of these observations commonly relate to $<10\%$ of the biomass while most of the biomass is attached in the formation and pack material out of sight (and out of mind) from the camera.

Given that most of the physical biofouling occurs out of sight of the camera (beyond the screen slots, perforations, or fractures) the importance of this fouling can often be ignored or, at best, only partially acknowledged. Risks from microorganisms growing in these attached (but invisible) communities range from plugging (loss in transmissivity through the biomass plugging the voids) to corrosion

and the degeneration of water quality. To locate and recover these microbes from these attached sites away from the borehole the environments where they are attached have to be impacted to cause these organisms (at least some of them) to detach and be carried in the water flow so that they can be recovered in samples. Under these conditions non-impacted wells may yield samples that have a low microbial activity while, once impacted, the samples would then contain much higher and more active microbial populations. Impacts commonly used are to change the pumping regime to include an extended period with no pumping. During this period some of the attached microorganisms are likely to free up from the attached growths (in search of a better and more stable home).

Changes (impacts) in these environments will cause this focus of biological activity around the water well to change. Commonly by shutting down pumping for an extended period of time then the biomass will begin to disperse. This then causes the redox fronts to move which now causes the attached biomass to become unstable releasing some of the microbial occupants from the slimes into the water. Once pumping is started again these released microorganisms move through the formations on the water flow into the borehole and up through the pump and pipes to the sampling port. During the pumping of the produced water it would, in turn, carry released microorganisms from the different biomass sites around the well upstream of the sampling site which means there is always an inbuilt loss of the precision created. Commonly the down time for sampling a producing well should be 1 week but practicality and demand may end up reducing this to an overnight period without pumping or just even for a few hours. Generally, the flushing and sampling of water for the released microbes can go on for two or more days but the most critical time is the first 2 h after the start of pumping.

L Application of BART Testers

L.1 USE OF BART TESTERS

One unique feature of the BART tester is that it automatically generates a vertical redox gradient with the oxidative region sitting above the redox front and the reductive region below. At the same time, the selective nutrimental chemicals diffuse upwards creating a multiplicity of different environments where some of the microbes in the sample may then be able to become active.

For the BART system testing of a treated well, assessment of the impact of any applied treatment should be delayed until after a period of biological stabilization has been reestablished in the well. For a new well this would be after the development of the well and for existing wells that have been treated it may take up to 8 weeks before this stabilization has occurred. Factors that should be considered in determining the time for evaluating the treatments on the biological fouling of the well are:

1. Ideally the treatment should have been undertaken at least 8 weeks before the post-treatment evaluation. Experiences have found that a minimum of 2 weeks is essential to achieve any level of precision with 4 weeks preferred if 8 weeks is not practical to the operational management of the well.
2. Specific capacity (Q/s) should be stable ($\pm 1\%$) and reflect improvements that could be related to the effectiveness of the treatment.
3. Water quality parameters should be stable and reflect improvements associated with the significant removal of elements that would have been associated with the removal of the plugging condition from the well [e.g., iron concentrations in the product water should have fallen since the biomass reforming around the well would now be filtering out (bio-accumulating) any iron moving towards the well in the groundwater].
4. BART systems should exhibit longer time lapses and lower predicted active cell populations in proportion to the effectiveness of the treatment. Suitability of the different BART systems to detect the effectiveness of particular treatments is dependent upon the environment in the water well.
5. It should be remembered that no well is sterile and all wells are subject to biological fouling to some extent and that any treatment, in order to be effective, needs to ensure that it addresses the control/disruption of that

biomass impacting the well and not just the normal background microbes always present within the groundwater for that well.

Treatment effectiveness can be determined at least in part by the impact of the treatment. Successful treatments can impact on the time lapses making them longer in the event of a successful treatment and frequently the reaction patterns observed will also change.

It should be noted that the iron related bacteria (IRB-BART) generates the more complex reaction patterns of the various BART testers. Additionally, the IRB generally are not the most active group of bacteria dominating the well. Their importance is more related to their impacts on water quality and secondarily on plugging risks. There is a need to determine the nature of microbiological challenges to a well when treated. Treating a well that is losing production/specific capacity as a result of biological events has the additional challenge that there will be a need to shock, disrupt, and disperse the biomass that is causing the problems within the environment of the well. The very nature of the shock, disperse, and then remove these dispersed growths treatments can now leave the surfaces clean again and unfortunately available for recolonization. It has to be understood that any treatment applied to a well is limited in its effectiveness to the regions of impact made by the treatment and beyond that impact zone there would likely be an initial zero effect. Additionally, such treatment impacts are limited to the environment of the well itself and the immediate regions outside the well and in the impacted aquifer.

In treating a well it can be expected that all of the treatment fluids applied to a well will tend to follow the lines of least physical resistance to flow which means the treatment around the well would be influenced by the movement of the fluids along those flow paths. This could result in geometrically disorganized treatments because of the extensions to the treatment zones out, and around. The surrounding aquifer environments will reflect these disparities. When production begins again for the well then it can be expected that the pathways of groundwater into the wells will also at least in part, reflect these opened passageways and porous media. During the immediate post-treatment monitoring for specific capacity it may be found that the results can often become erratic as the microbes recolonize the treatment-cleaned surfaces. This is symptomatic of the reemergence of microbial biofouling and the realignment of physical particles disrupted by the treatment processes. As a result of these treatment and post-treatment events it can be expected that precise and reliable data on the effectiveness of a treatment cannot be determined until after the well environment has stabilized again following treatment. This may take as long as 8 weeks.

L.2 FUNCTIONAL ADVANTAGES AND CONCERNS OF MOLECULAR AND GENETICALLY BASED DETERMINATIONS OF MICROBIAL PRESENCE AND ACTIVITIES

Microbiology today is preoccupied with the ability to determine, by various biochemical and/or molecular means, the specific types of microorganisms. This of

course has relevance to detection of specific pathogens so that the source and risks can be quickly established and managed. In water wells, infestations are not the result of a single species of microorganism but are more the result of the activities and interactions of many microbial communities that could collectively include as many as a hundred or more species. Medical Science has yet to acknowledge health risks associated with microbiologically derived community challenges. In the determination of the cause of biofouling within a water well environment and the surrounding aquifers, it becomes important to determine which communities are active and also which are likely to be causing significant problems to the wells production.

In the determination of cause, risk, and effective recoveries of water wells, it is important to use fast and economical microbiological test methods that can be applied in the field to determine the activities of various microbial communities active in the well and surrounding aquifer. Biochemical and molecular testing including the identification of genomes, commonly requires sophisticated laboratories, and involve expensive and time consuming methods. At this time there appears to be no simple low-cost molecular-based tool that could reliably and economically be used to determine the presence and activities of a broad spectrum of microbial communities associated with water well biofouling.

M Evaluation of Risk Potentials Relating to Specific Capacity, Plugging, Corrosion, and Health Risks

M.1 INTRODUCTION

Specific capacity is a very important monitoring tool. There should be a zero tolerance for any significant losses in specific capacity where the groundwater static level remains constant. If the specific capacity (Q/s) is falling while the water table remains stable then that is an early indication that could be an indication of some type of biofouling occurring. Biological activity reaction tests (BART) analysis of the well waters allows plugging to be monitored through sequentially sampling the pumped water from the well. By conducting BART tests prior to, and then again after a well regeneration or preventative maintenance servicing treatment, it is possible to determine the effectiveness of that treatment. There are a number of important considerations:

1. The time for sampling after a treatment has been completed is critical. This is because the impact of a successful treatment can be very disruptive. This would mean the treatment would have killed some microbes, flushed other microbes out of their "safe" environments, and cleaned many of the surfaces in, and around, the well. However, immediately outside of any "kill" zone (where virtually all of the microbes would have been killed and the surfaces scoured relatively clean) then there is a "disrupt" zone where there are considerable numbers of microbes still present and active but not in their normal habitat. Beyond those "impacted" zones (the "kill" plus the "disrupt" zone) would still lay communities of microbes that had not been substantially impacted by the treatment (non-impacted zone). Even a very effective treatment cannot extend out into the non-impacted zone since it extends outwards into the vast body of that groundwater resource that lies beyond the influences of the well.

2. The goal of an effective treatment is to significantly recover the specific capacity of the well, in part, by removing the microbially generated plugging structures. Such a treatment will, by the nature of its success, have generated cleaned surfaces (onto which microbes may now attach and form fresh biofilms), increase the available organics as food for the microbes (through the disruption of the plugging structures), and create the potential for sudden microbial invasions of the treated regions in, and around, the well. It may therefore be expected that there will be a significant "burst" in microbial activities within the treatment impacted zone after treatment. Such activity "bursts" are likely to give very high levels of recorded activity in BART testers applied to post-treatment water samples collected during times when there is an active "burst" occurring.
3. The "burst" phase when the microbes are extremely active generally continues for at least 14 days (2 weeks) but can last for as long as 42 days (6 weeks). It is therefore recommended that post-treatment water sampling is not taken until 56 days (8 weeks) after a well has been treated to assure that the initial "burst" of microbial activity has subsided and that the samples reflect more the background microbial activity now occurring in the well.
4. While changes in specific capacity may be reliably observed as soon as 7 days after treatment, the impact on the microbial communities in, and around, the well may not be accurately determinable until 8 weeks after the treatment because of the likely reactive "burst" of microbial activity following the treatment within the impact zone.
5. Sampling a well in which most of the microbes are living attached to surfaces means that, unless proper practices are applied, water samples gathered for testing may not truly reflect the degree of microbial activity within the well. As a consequence, it is necessary to apply some disruptive practices to the well in order to encourage microbes to detach, enter the groundwater and hence, be pumped from the water well to form a suitable sample. This disruptive practice involves breaking away from the normal operational mode for the well to a sufficient extent that the at-site environment for the microbes shifts sufficiently to trigger the microbes to move up, away, and relocate. For an operating well (e.g., 70% of the time in production during any given 24 h period) the easiest manner to achieve this low level of disruption is to take the well out of the production cycle for at least 24 h. This causes the environment around the well to now become more reductive causing with some of the attached microbes to now relocating into the groundwater. The minimum disruptive times that can have an impact causing a relation response are from 8 to 24 h (preferred).
6. Sequential sampling over timed intervals from the continuously flowing water from the well can be used to determine the approximate positions of the various microbial groups around the well. Using the BART-SOFT version 5.0 using the zones of interrogation projections program can allow a pictorial image of the positions of these various microbial communities to be determined.

7. Microbial activity levels are determined in the BART testers by the time lapse that occurred to the observation of the first positive declaration of presence. This can be undertaken visually by examining the reacting BART tester on a daily basis or for the HAB-BART system employing the reader then there is an automatic monitoring of the tester and declares a positive with the time lapse. Most of the BART testers can also be monitored using the V-BART-READ system in which an approved digital camera (e.g., Cannon S3 or S5) logging is used to monitor the reactions and generate a time lapse.
8. It is possible to project the dominant microbial community detected in the BART tester by the reactions displayed. Changes in the data generated by the BART testers observed following a treatment can then be used to determine whether there have been significant impacts caused by the treatment on the microbes associated with the biofouling.

M.2 SUSTAINABILITY IN WATER WELLS: SIGNALS OF SUCCESS AND WARNINGS OF DOOM

Drilling and developing water wells in order to produce predicted specific capacities are a challenge particularly to make the user smile "iron"-ically. Most consultants and drillers leave a site with the feeling of a job well done. As for the wells, they now slowly begin to "age" and the water quality declines with production drops following a pathway dictated by that environment and its use while it should be remembered that the wells do not come with a maintenance manual to guide the operator through keeping the well serviced. In the past, these failures have been placed firmly in the lands of geology and chemistry. Today it is becoming more apparent that water wells can become infested with microbes growing within, and around, the well to gradually throttle down the wells production while at the same time causing the water quality to fail. Traditionally a well was considered to be clogging when these symptoms of failure were observed. The cause was commonly put down to the perching of materials such as clays, silts, or sands (fines) around the well. The advent of routine camera logging revealed that there were often encrustations around the well particularly on the well's slots. These encrustations were first thought to be extensions of the chemical action (e.g., ochres) that had been associated with the gradual hydraulic failure of the well. In the last 30 years it has become increasingly evident that water well failure is often not so much a chemical or physical phenomenon but more a result of biological activities. Critical microbial groups now commonly associated with these types of water well failure include (in the rogues gallery):

1. Sulfate-reducing bacteria (SRB) that can cause corrosion and generate rotten egg-type odors and black slimes.
2. Slime-forming bacteria (SLYM) that can cause plugging, turbidity (cloudiness), slime build up, taste, odor, and encrustations to form.
3. Iron-related bacteria (IRB) that can cause plugging, yellow waters, fluctuating iron and/or manganese concentrations in the water with iron-rich encrustations, nodules, and/or scaling on surfaces.

4. Heterotrophic aerobic bacteria (HAB) that can cause slime formations, deteriorating taste and odor, particularly when wells are being polluted with organic solvents and hydrocarbons.

The next challenge once the guilty bacteria are detected is how commonly do these events affect the life span and efficiency of a well? If the basic premise is that geochemical events are the basis of a clogging then biological events are the basis for plugging. It is now necessary to ask which of these two factors most severely influences the life span of wells? This may be answered by examining the average life span of a well. In two recent studies, the life spans of wells in the Three Hills M.D., Alberta was placed at 15 years while in the Mount Hope R.M., Saskatchewan a similar survey revealed a probable life span of 18 years. Essentially these wells were all in the category of minimal maintenance and on-demand use. Such an attitude considers that the wells are disposable commodities to be replaced when they can no longer generate an adequate quality or quantity of water. In the current researches, the basic idea is how to move water wells from being disposable "throw away" items (when they fail) to becoming sustainable "keep them going forever" items with a prolonged life span. Moving from the disposable to the sustainable is a major challenge to the water well industry. It means moving from a mindset of minimal maintenance limited almost exclusively to addressing mechanical failures; to a mindset of preventative maintenance servicing and proactive treatment scenarios where the water wells are serviced on a routine basis in much the same way as a car or truck. Tell tale signs of failure include the following common observations:

1. The specific capacity has fallen by more than 5% below the original values observed when the well was newly developed.
2. The bacteria are becoming more aggressive with the time lapse (days of delay) dropping by a day or more.
3. Water quality processes particularly with respect to turbidity, iron and/or manganese; or taste and color degenerates.

Monitoring the specific capacity of water well has the same value to the well operator as taking the patient's body temperature has to the physician diagnosing a sickness. Any fall in the specific capacity caused by clogging or plugging should be taken as an early warning sign and a preventative maintenance servicing needs to be started immediately. This may be a problem when the management does not appreciate the needs to spend maintenance funds in order to maintain well production. At this time the water well is on the slippery slope of slime to failure. If specific capacities continue to fall then the hope of recovering the well to its original specific capacity also goes down. The "grey zone" is a 15%–40% loss in specific capacity. Here, there remains a reasonable chance of returning the well to its original specific capacity while wells that have failed by more than 40% do not offer that same hope (certainty). Generally, under the more serious circumstances of severe plugging the most that can be expected is a 10%–30% recovery if the treatment has been at least partially successful. Losses in specific capacity of more

than 40% in a well can be commonly viewed to be well down the "slippery slime slope" to failure and abandonment.

Moving water wells from disposable "throw away" items to sustainable and reliable sources of water means that preventative maintenance servicing has to begin with the design of the well and be practiced from the time the well goes into service. Delays in bringing a well on-line can also create problems since "an idle well is a fouling well" (this goes frequently unrecognized). It should be remembered that the only yardstick to measure in, an effective manner, the recovery of a well is by comparison to the original specific capacity (Q/s) when the well had been developed. The maximum improvement that can be achieved is 99.9% towards the original Q/s assuming that the original Q/s was accurate and the water table has not changed. Some practitioners of well regeneration use the Q/s from immediately before treatment and take the percentile gain in performance as that increase in Q/s caused by the treatment to the Q/s from immediately before treatment. Using this method, it is possible to get claimed improvements in the order of much greater than 100% (of the pretreatment Q/s). This does not relate to the true measure of improvement which should always be based upon the original development Q/s the well when it was first put into service. Any contractor who claims exaggerated recoveries based upon the pretreatment Q/s is not taking into account the true nature of the well. For example, a well that was operating at 40% of its original Q/s and was brought back to 80% has therefore shown a 40% improvement in Q/s through rehabilitation. Using the pre-rehabilitated Q/s as the benchmark (baseline) would mean that there had been a 100% improvement in the specific capacity claimed. From the quality management perspective, only the original Q/s can be used as the only acceptable benchmark since then all data will relate to a common source (the original post-development Q/s). Only this would allow effective cross comparison of the various treatments applied to that well.

Sustainability has now become an accepted part of the fabric of life. In applying this concept to water wells a whole new set of challenges emerge. These range from the fundamental shift in attitude that would occur when moving from the drilling of replacement wells to the regeneration and preventative maintenance servicing of all existing wells with the aim of significantly extending their life span. Capitalization in the end would, under these circumstances, shift from drilling new wells to the treatment of existing wells. One challenge that remains is to build the same level of confidence into well regeneration as exists in well drilling. Locating and drilling a well is firmly based in the knowledge of local hydrological conditions and the geological formations. At the same time it is equally important to get information on the microbiology of the formations and other local wells in the same formation. If there are aggressive bacteria already within the formation then there is a greater probability for plugging and other bacteriologically influenced problems to occur. In examining well fields for bacteriological challenges the BART testers can be used to diagnose dominant types of bacterial infestations. These are listed in the next paragraph (see also Appendix B).

Sulfate-reducing bacteria are often the bacterial group of the most concern (Kings of Corrosion) due to their ability to grow at focused sites and cause electrolytically based corrosion of metals as well as the production of black slimes

and rotten egg odors. Slime-forming bacteria bacteria are the next (Queens of Sliming) causing heavy slime formations and encrustations. Iron-related bacteria are the "Jacks of Rust and Crust" and are regular participants in any iron-rich plugging of water wells. They are very difficult to control once they have become established within a well. Heterotrophic aerobic bacteria are the Workers (or perhaps the Jokers!) often biodegrading many of the organics including solvents and hydrocarbons. These commonly become dominant in water wells that have become impacted by hazardous wastes incorporating organics such as petroleum hydrocarbons or solvents.

Each of these bacterial groups work with the others in different ways so that each well, as it biofouls, develops unique signals requiring different treatment challenges which means that no one size fits all and consideration has to be given to each well separately. Laboratory investigations need to be undertaken to determine both the nature and the vulnerability of the microbes at the various plugging sites in and around a well. There is therefore a need to introduce and understand the roles of the various microbial communities in the biofouling of wells is becoming a significant part of the treatment and management process for which the protocols are now being developed.

Selecting a regeneration and maintenance management servicing procedure for a well prone to plugging does not have a firm basis in historical practices mainly because plugging has for many years been considered to be a relatively insignificant event. As a result it is often lost in the dynamics and practice of denial countered by the urgent need to replace the well if the demands for water exceed the ability of the well field to supply. As the science of well plugging moves forwards the practices to control plugging should start before the problem becomes too serious. If practiced effectively the wells would become more sustainable and less a disposable item. In well treatment and preventative maintenance practices, it has to be remembered that all that can really be achieved is a delay in the inevitable event (i.e., the water well failing). This event occurs when the well biofouls, plugs, and loses production capability. Sustainability practices are a means of delaying inevitability and, at the same time, improving the performance of the well for a longer managed period of time.

N Regulatory Considerations Concerning Phosphorus

In the regulatory consideration of water wells, there are a number of areas of regulation well established. These relate particularly to societies use of wells as sources of "safe" available potable water. Considerable attention is paid to health risks from microorganisms (particularly the coliform group of bacteria). Additionally, potentially hazardous chemicals from organics such as the trihalomethanes and benzenes to inorganics such as arsenic and chromium have also been considered. Broadly sweeping regulations protect society from significant risks that these chemicals can present. Fewer regulations are however in place to protect groundwater systems (including water wells) when compared with the surface waters (we protected the consumer but not the source!). Water well treatments, by their very nature, can include the use of physical, chemical, and even electrolytic methods to rehabilitate a well. Phosphorus has been one of the chemicals commonly applied to wells in various forms but usually as a phosphate or acid. Consequently, phosphorus has become well recognized as a cleaning agent.

Phosphorus dominated the detergent industry up to three decades ago because of superior ability to clean surfaces. It was with the recognition that phosphorus was a major nutrient which supported the algal blooms in surface waters that the downside of this element was recognized. Subsequently, there was a banning of phosphorus in detergents to reduce the risk occurrence of pollution in surface-waters associable with the use of these detergents. However, blooms in water wells are not so easily viewed, recognized, or made quantifiable except through indirect symptoms such as significant drops in the specific capacity and failing water quality. These failures are frequently linked to biological growths occurring not only down the borehole but also outwards into the producing formations. Parallel conditions may therefore exist where blooms in water wells may become more controlled by the banning of phosphorus from treatment options applicable to plugging water wells.

In science, applications of phosphorus are commonly used to stimulate growth. In water wells such growth could also accelerate the rate of failure assessed through both specific capacity and water quality. Forms of phosphate that could significantly impact a water well causing exacerbation of biofouling problems is not limited to phosphate (PO_4) but also includes all organic and inorganic forms of phosphorus including polymerized forms and phosphoric acid. Advocates for the application of phosphorus to water wells assert that, as a part of cleaning strategies, treatment

effectiveness is enhanced since all of the applied phosphorus is totally recovered during the later stages in the treatment process.

Reality dictates that the applied phosphorus (of any type) can readily be converted by the biomass surviving the phosphates and polyphosphates treatments can now accumulate within the biomass for later growth. This would be particularly probable in groundwaters passing through organic-rich zones such as those that would occur in alluvial river deposits. Such materials are likely to become more biologically active during treatments causing shifts in the in situ oxidation–reduction potential that could trigger extensive biological activity and stimulate greater levels of phosphorus accumulation. In the event that a strongly acidic phosphatic product was applied such as phosphoric acid then the biomass could still accumulate the phosphorus through a buffering action within the biomass. Such events as those described above would result in the waters produced after treatment having a zero phosphorus balance, not because all of the phosphorus applied has been recovered during the treatment process, but because the phosphorus has now become locked up into the biomass and would now be readily available to stimulate a downhole bloom.

It is very logical that the ban on the use of phosphorus in detergents should be extended to include all well treatment agents containing any significant amount of phosphorus. In the ideal treatment, all of the applied phosphorus is removed along with all of the indigenous phosphorus from the biomass (which would mean that all of the biomass had been effectively disrupted and dispersed from the biofouled well). At a very minimum the treatment should recover all of the applied phosphorus so that there would be no additional inputs of phosphorus resulting from the treatment that could cause an additional microbiological bloom around the water well.

Such regulatory initiatives could include the banning of phosphorus as a significant component in the treatment of water wells. This would now act to lengthen the expected life span of water wells through reducing the future potential for biofouling. The outcome of such a ban would parallel the effective banning of phosphorus-based detergents to control blooms in surface waters. Here the outcome would be the controlling of "blooms" of iron-related bacteria and other microorganisms in the groundwater. This initiative would then materially prolong the active life of water wells.

> Many iron-ores are highly phosphoric. This phosphorus must of necessity be organic in origin, and the possibility that a certain portion of it was derived from the remains of organisms which possessed the same physiological attributes as the iron-bacteria is not altogether excluded.
> Ellis, D., 1919, *Iron Bacteria*, London, Methuen & Co. Ltd., 176.

O Applications of Heat as Component in Regeneration of Biofouled Water Wells

It is well known in the science of chemistry that the use of heat will accelerate the rate of most chemical reactions. It is also a well known fact in the science of microbiology that microorganisms will only function over a limited temperature range. These two features are employed in the patented blended chemical heat treatment (BCHT™) process. Here the chemistry of the treatment (commonly employing non-phosphate biostatic detergents along with significant pH shifts of greater than 4 pH units) has a greater impact when the temperature is elevated by greater than 40°C. At the same time as the heat speeds up the chemistry, the thermal gradients created by the inputs of heat kill off or traumatize the vegetative microbial cells within the target zone. Here, death rates can commonly range from two to four orders of magnitude. This created thermal gradient will extend from the borehole (if that is the site for the application of the heat) until at some distance (such as 20 ft) where there is now a zero thermal impact on the background temperature. Within the thermal gradient zone there is normally a period of 1–3 days where the temperature has elevated by the heat treatment. Close to the borehole such elevations would normally traumatize the microbial community with few survivors. Further out along the gradient, the temperature gradient would be less severe and there could theoretically be some microbial activity stimulated by the elevated temperatures with nutrients becoming available from the dead (killed) microbial cells emanating from the heat-treated sites down the borehole. Since the thermal gradient cools down very quickly there is only a brief window for such enhanced growth. If the well has been properly treated then much of the dispersed material should have been removed from the well minimizing the potential for microbial growth. The claim that these elevated temperatures do stimulate microbial growth has credibility in theory but, in practice, the thermal pulse associated with the heat treatment is too brief to allow an effective growth of microorganisms in a well environment. Such a growth would have to involve to phases (1) an adaptation of the bacteria to the elevated temperatures which might commonly take several days or weeks and (2) growth which would normal involve a further lag as the bacteria now

begin to grow. It should also be recognized that the normal generation times for the indigenous bacteria in a water well has to be measured in days, weeks, or months while the cooling off period for a well would not extend beyond 4 days. All of these considerations therefore act to reduce the probability of a sudden burst in growth of bacteria in that relatively short period of time when the well temperatures are elevated. Increases in bacterial populations following heat treatment are most likely the result of the enumeration of dispersed survivors that are now moving through the impacted zones in the product waters. These are commonly a result of transient dispersion and do not reflect sudden growths of microorganisms in the heat-treated zones.

From the regulatory perspective, the application of heat to a well and the surrounding groundwater is much less likely to generate any unacceptable chemical daughter products that would have a much greater effect on the microorganisms and, in particular, the coliform bacteria. Here, application of heat could be viewed from the regulatory perspective as a much safer treatment option than the straight use of chemicals without heat. Without heat, the amount of chemicals that would have to be employed would have to be that much more in order to achieve an effective dispersion of the plugging biomass. Therefore, minimally, the heat should reduce the amount of chemicals that have to be employed.

P Application of Vegetable Oil as Substitute for Hydrocarbon-Based Oils to Lubricate Pumps

Hydrocarbon oils have long been used as lubricants in pump motors but do leak into the borehole and can degrade there. This may lead to too numerous to count bacterial numbers or very high BART aggressivities (activities, short time lapses) that could cause the regulatory rejection of the well. Hydrocarbon oils have been replaced by vegetable oils with one of the selling features being that they are "safer" and biodegradable. It is a logical extension of that environmentally friendly argument that raises the question "what happens to the microbial biomass that has degraded the vegetable oil?" The answer has to be that the vegetable oil can radically stimulate the growth of microorganisms' down hole and thus present a greater biofouling and health risk which could become a regulatory concern. For the hydrocarbon-based oils, degradation is generally much slower because of the complex nature of the polymers in the oil. Both types of oil will break down but the more degradable vegetable oils will tend to degrade faster and potentially stimulate greater levels of biofouling activities. Any spillage of oil into the borehole is also likely to present potentially significant biofouling events. In the regulation of these oils it has to be recognized that both oil present risks to the microbiological health of the well with the vegetable oil, in all probability, having a more immediate impact than the petroleum-based oils.

Q Frequently Asked Questions

Below is a list of common questions asked by well owners that could be relevant to microbiological events occurring in, or around, water wells.

Q.1 WHAT ARE COLIFORM BACTERIA?

Coliform bacteria are always in animal and human feces and when you find them in water it may mean that feces are probably also present. If such feces are present in water then there is also a possibility that disease-producing microorganisms (pathogens) may also be present. In regulations, it is the presence of coliform bacteria that raises the health risk level in the water to a level where there may be harmful pathogens in the water unless disinfection or boiling is adequately applied to the water.

Q.2 ARE COLIFORM BACTERIA PATHOGENIC (DISEASE PRODUCING)?

No, the coliform bacteria are mostly bacteria that normally live in the gut of healthy humans and animals. There are some exceptions, in particular, *Escherichia coli* 0157 that is able to cause serious gastro-enteric infections. It has to be remembered that the coliforms are an indicator group whose presence in water signals that there could be a potential health risk from other types of bacteria and precautions need to be taken to reduce that risk.

Q.3 WHAT IS THE DIFFERENCE BETWEEN TOTAL COLIFORMS AND FECAL COLIFORMS?

Total coliforms embody a broad range of bacteria that can occur normally in animal and human guts and, for some, also in the environment. The fecal coliforms are more specific and are related to the types of coliform bacteria that are found just in the human gut and feces. The dominant bacterial genus in the fecal group is *E. coli* (in the short form).

Q.4 WHICH OF THE TWO COLIFORM GROUPS PRESENT THE GREATEST HAZARD IN DRINKING WATER?

Undoubtedly, it is the fecal coliform bacteria since this group is much more specific to *E. coli* that is a major bacterial species in human and animal feces and therefore more likely to be present when there is human sewage or septic wastes getting into a water system.

Q.5 ARE COLIFORM BACTERIA ONLY FOUND IN WELL WATER AFTER CONTAMINATION WITH WASTES?

Most coliform bacteria arrive with fecal contaminated or septic wastewaters that could also include surface waters polluted with human, domesticated animal or wild-life feces. Some of the total coliform bacteria are also capable of growing in the borehole and around the screens of water wells. Fecal coliform bacteria generally do not survive and grow in or around the water well.

Q.6 ARE WATER WELLS STERILE WHEN NOT CONTAMINATED WITH COLIFORM BACTERIA?

There is a very rich and diverse microbial growth in and around water wells extending outwards into the aquifer. This growth includes a wide range of bacteria and also some molds (fungi) mostly as spores.

Q.7 WHAT DO THESE OTHER MICROORGANISMS DO TO A WELL?

There is both good news and bad news. The good news is that the microbes will actually act as a biological filter and clean up the groundwater flowing into the well, taking out organics (including solvents and petroleum hydrocarbons) and metallic cations such as iron and manganese. The bad news is that the microbes grow to form thick slimes (biomass) that become unstable and so that the accumulated organics and metals are suddenly erratically released. These microbes can also then start to impact on the well's water quality and quantity.

Q.8 HOW DO THESE BACTERIA CAUSE A WELL TO LOSE WATER QUALITY AND QUANTITY?

Water quantity is lost since the slimes generated by the microbe's plug up the formation and pack materials around the well. This causes the well to lose specific capacity (Q/s) and is called biological plugging of the well. Water quality is lost as the slimes break away from the biomass into the water as biocolloids carrying some

of the accumulated organics and metals in these particles. This will be seen as erratic and even sudden reductions in water quality that may even cause the water to become more turbid and have a much unacceptable chemistry.

Q.9 CAN THESE MICROBES ALSO PHYSICALLY DAMAGE A WELL?

Yes, once the slime plugs have formed then some of the bacteria in the slime can begin to generate acids and hydrogen sulfide gas. This gas reacts chemically to create black sulfide-rich slimes in the well and can be released to give that typical "rotten egg" odor that can affect consumer acceptability. This gas also starts up corrosion processes that can perforate and even destroy steels. Acids can drop the pH and cause metals and concretes to corrode. Wells can therefore become physically damaged by the corrosion of metal fittings and casing.

Q.10 WHAT HAPPENS IF WE DISINFECT WATER WELLS?

Disinfection means what it says: dis-means to dispel or remove; -infection means some infectious agent. Disinfection therefore removes (by destroying) these infectious agents thereby reducing the health risk. When a well is disinfected, it does not destroy all of the microbes but only those that may be infectious to humans. The coliform bacteria are commonly used as the guinea pigs since disinfection is only considered successful when all of these coliform bacteria are destroyed. Not all of the other bacteria are however destroyed by disinfection and plugging and corrosive events caused by the microbes may not even be slowed down!

Q.11 SHOULD I USE CHLORINE TO DISINFECT A WELL?

One of the classic disinfection treatments has been the use of some form of chlorine. It is known that chemicals containing chlorine do have a greater action on the coliform bacteria and so can effectively control many of the potential pathogens that can present a health risk. Almost for the last century, these chemicals have been used as the main stay of many disinfection practices. In the last two decades however, chlorine has become linked to the potentially carcinogenic trihalomethanes and the search has been ongoing for suitable replacement disinfectants. Chlorine can still be used to disinfect wells but checks should be made to determine the local regulatory conditions. One of the advantages of chlorine is that it is very effective and can leave a little residual disinfecting activity when there remains some free residual chlorine in the water. Slimes can pose a problem since the biofilms will protect the microbes from the impact of the treatment. Often chlorination will cause the slimes to contract (giving the well a much improved specific capacity for a short while!) but the slimes can quickly rebound in a matter of days or weeks to return the well to its previous plugged state.

Q.12 IF I CANNOT USE CHLORINE DISINFECTANT, WHAT OTHER DISINFECTANT CAN I USE?

There is a range of other disinfectants but these tend to use different principles and be less selective for the coliforms and pathogens. The major groups are listed below

1. Oxidizing agents based on ozone and peroxide. These chemicals generate an extremely oxygen-rich environment in which free hydroxyl radicals form and these can destroy microbial cells in a non-selective manner.
2. Acids. There is a range of acids that have been used to disinfect and clean wells. These range from muriatic* (hydrochloric), acetic (vinegar), glycolic (hydroxyacetic) to sulfamic* acid. Inorganic acids (*) tend to be much more aggressive on slimes and carbonate scales but generally do not penetrate the slimes so effectively. The others tend to also act as inhibitors of microbial activity and may also have some detergent effects through cleaning the slimes off the surfaces in the formation.
3. Surfactants, dispersants, and penetrants. Working like detergents, these chemicals actually can penetrate the slimes and break apart the molecular strings that bind the slimes together and hold the water in place. There is a wide range of these chemicals that have very different characteristics and care should be taken to select the right chemical to meet the needs. In simple terms, surfactants tend to work over the surfaces of the slimes causing gradual collapse, dispersants cause the slimes to break up steadily and disperse while the penetrants "tunnel" into the slimes to destabilize and disrupt.

Some of these chemicals can be bactericidal and kill off the microbes within the slimes. In selecting one of these chemicals, it is prudent not to use the one that will "feed" the survivors afterwards with nutrients such as phosphorus-based chemicals.

Q.13 IF MY WELL IS PLUGGING WITH MICROBIAL SLIME? CAN I USE HEAT TO HELP DESTROY SLIMES?

In biofouling water wells, the microbes living down in the slimes, plugs, nodules, and encrustations are used to growing at a pretty constant temperature. They can protect themselves to some extent just by their slimes, scales, and crusty coatings. Often it is difficult to get chemicals to penetrate completely through these coatings even when penetrants are used. This is why it has been found very effective to add heat at the same time as the chemical treatment. With hot chemical solutions and even steam it is possible to heat up the water in the borehole by at least 60^{o}F (15.6^{o}C). That temperature penetrates the slimes, shocks the microbes, disrupts the growths, and kills many of the microbes. An example is the patented blended chemical heat treatment (BCHT™) process. Here the heat is delivered with disinfectants and penetrants that unravel the molecular threads binding the slimes

and growths together. This is followed by a hot acid wash that takes the heat and the treatment back out into the formation. All of the biofilms in the zones affected by the heat and the various chemical solutions start to collapse and the surviving microbes become dispersed into the hot solutions. Once the microbes causing the plugging and corrosion have been dispersed then they are pulled out of the well usually by surging with air forced down the borehole.

Q.14 I HAVE HEARD THAT HEAT SIMPLY PUSHES MICROBES FURTHER INTO THE FORMATION AND CREATE AN EVEN BIGGER PROBLEM. IS THAT CORRECT?

No! heat delivered with various chemical solutions down the borehole creates hot zones in, and around, the well in which the microbes are disrupted with many being killed. When the well is surged to remove the debris much of these disrupted bacteria are removed. The survivors still down the well have been so shocked by the treatment that it takes them a long while to reestablish biofouling. One thing microbes love to do is cannibalize all dead cells still down there along with the nutrients that may have been released. This is one of the reasons that polyphosphates are so bad because this helps to "kick-start" this new colonization of the well. When the microbes grow they will do so on the fringes of the treated zone where there is very little oxygen but nutrients are coming in with the groundwater. This fringe is where the environment moves from oxidative (because of oxygen) in the well to where it is reductive outside of the well. This fringe is called the "redox front" and in the laboratory we have found that 80% of the microbes often collect right there and so that is where many of the problems can occur.

Q.15 CAN I TAKE A WATER SAMPLE AND SEND IT AWAY FOR TESTING FOR REDOX (ORP) TO SEE WHERE THE MICROBES ARE?

Of all the tests for water, redox is one of the most difficult. Remember that when you take a sample you are likely to bring the sample into contact with air. This will cause oxygen to enter the sample and make it more oxidative. To do a redox test (also known as the ORP) the test needs to be done preferably right at the same time as the water sample was taken. Mailing a water sample to a laboratory for a redox test is basically impossible. The reasons are that if you did not allow air to enter the sample (the presence of air would cause the sample to go oxidative when it is shaken up during transit to the laboratory) then microbial activity might end up making it go more reductive because of the activity of these microbes. Either of the results obtained from a laboratory would therefore have no value since it would not reflect the true redox value or ORP for that

water sample that would have been measured at the site of collection. The only value would be to the laboratory charging for the test!

Q.16 HOW CAN I TEST WATER FOR BACTERIA THAT MIGHT CAUSE BIOFOULING OR CHECK A WELL TO SEE HOW SAFE THE WATER IS TO DRINK?

Making sure that the water is safe to drink is a real priority. The standard test is the coliform test and there are a range of tests out there, some of which can be performed by a qualified person right at the well. Pretty well all environmental and water analysis laboratories will undertake coliform testing for both the fecal and the total coliform groups. Remember these tests do have to grow the coliforms at blood heat and it takes at least a day to get a result and the numbers in a good water sample is zero detected in a 100 mL water sample (that is about a quarter of a pint). If you do have coliforms in your well then remember there is very little tolerance in the regulations for their presence and the well will need to be disinfected and tested regularly. It may only be used for drinking once no coliforms are detected. Check local regulations on the exact requirements but testing for coliforms needs to be done regularly with an approved system—your health is at risk if there are coliforms in the water. Other microbes are common in water and grow there quite naturally but they do also cause problems. These problems range from a failing well plugging up to equipment corroding, water quality declining with taste, and odor problems becoming unacceptable. Some of the bacteria involved in these events include

1. Iron-related bacteria that thrive on iron and grow on the oxidative side of the redox front. They develop brown, often crusty slimes, produce yellow and red-brown waters and plug up the well (particularly the pumps).
2. Sulfate reducing bacteria love to grow in reductive conditions and they often use sulfates from the water and generate hydrogen sulfide (the rotten egg odor). The water may go gray or black, generate horrible smells, and start to cause the corrosion of metal fittings.
3. Slime forming bacteria often dominate the redox front but can grow deeper out away from the well. They develop heavy slime growths that are usually white or gray in color and can cause the water to become very turbid.
4. Heterotrophic aerobic bacteria (HAB) are very adaptable and generally dominate in wells that contain more organics or have been polluted with organics such as solvents, petroleum hydrocarbons, and septic wastes. They generally do not produce thick slimes but can grow very quickly causing the water to become turbid and sometimes begin to smell. The most common smell is that of fish since one the HAB bacteria (e.g., species of *Pseudomonas*) often produce these smells.

Not so many laboratories test for these bacteria other than coliforms since they are not directly linked to a health risk but they do pose very significant risk to the well.

Testing for these bacteria can be performed in the field using presence/absence techniques using the BART™ testers. Here the aggressivity (activity) of the bacteria is measured. If the bacteria become more aggressive with subsequent testing, it means that the biofouling is getting worse and the well should be treated to reduce the effects of these bacteria. You can also send the sample away to a laboratory but it may be 2 or 3 weeks before you get an answer to your concerns. In sending a sample away for bacterial testing, the sample should be sent by the most rapid suitable means (e.g., courier service) since the microbes in the sample will be impacted during the transit time to the laboratory.

Q.17 IS IT TRUE THAT SOME EXPERTS SAY THAT THE BACTERIAL PROBLEMS IN GROUNDWATER ARE NOT SIGNIFICANT?

Some experts seriously believe that their products and services do control bacterial fouling in wells and so they would say that bacterial problems are not significant (and anyway they can fix it easily at a cost!) and the bacteria will not come back. No well treatment can sterilize water wells effectively and once the treatment is complete it is inevitable that the microbes will come back rapidly to seize back their homes and grow, grow, grow. Bacterial tests show that when this happens then those experts may not have quite so much control as they thought they had. The answer is to do the bacterial testing, check on the effectiveness of the treatment, and not to believe any expert until you are sure that the job has been done right and the well is back to its original production with acceptable water quality. Remember even if you can achieve effective treatments that the bacteria will come back and you have to begin a program of preventative maintenance servicing of the well.

R Glossary

Acid-fast. A staining procedure which differentiates out the bacterial genus *Mycobacterium* due to its ability to retain a dye under acid–alcohol washing. One species causes tuberculosis and another leprosy.

Acidophile. The name given to microorganisms which grow most efficiently within the pH range from below 1.0 to 5.5.

Acinetobacter. Gram-negative rod-like bacteria which become coccoid as the cells age. These bacteria are commonly found in surface waters, tailings ponds, and distribution systems. They are sometimes called the "weeds" of aquatic bacteria, and are associated with outbreaks of gastroenteritis and water-borne (nosocomial) infections.

Actinomycetes. Aerobic gram-positive bacterium which grows forming branching filaments (hyphae) and produces exospores. Can form mats similar to fungi.

Active bacteria. Bacteria being in a state of either growing or metabolically active. This occurs when the environmental conditions are favorable for the requirements of the particular group or groups of bacteria involved.

Adaptation. The intrinsic ability of microorganisms to adapt successfully to changes in the environment. Many microorganisms have the facility to adapt their metabolic processes to meet the needs dictated by changing environmental conditions (i.e., nutrient source change).

Aerobe. The name given to an organism that grows in the presence of oxygen. Growth may occur only in the presence of oxygen (obligate/strict) or may also occur in the absence of oxygen (facultative).

Aeromonas. Gram-negative, facultatively anaerobic bacterium related to the coliform bacteria (but not forming a part of that family). They are widely associated with diseases of a variety of fishes and reptiles. These bacteria can also be present in groundwater systems usually in very low numbers. The species *Aeromonas hydrophila* has been associated with outbreaks of gastroenteritis and is considered to be a nosocomial pathogen.

Aerotolerant. The condition whereby an organism can grow equally well whether oxygen is present or not.

Agar. A gel-like seaweed extract that has been used successfully since the late nineteenth century for the cultivation of some microorganisms. Over the last 100 years, there has become a reliance on agar-based media to culture microorganisms to the exclusion of those organisms that will not grow on agar.

Algae. Small single or simple multicellular plant-like organisms which grow normally in the presence of light by photosynthesis. They occur widely in freshwater and are related in population size to the degree of nutrients available to the water body. Some are capable of using organic materials for growth (heterotrophy) and may occur in wide bore or shallow wells and in deeper wells where there is an adequate organic nutrient base.

Anaerobe. The name given to organisms that can grow in the absence of oxygen. Many of these bacteria are known to be so sensitive to oxygen that the presence of even low levels (i.e., 0.1 mg O_2/L) will kill the cells.

Antagonistic effects. The detrimental impact one organism has on the survival and/or growth of another organism through the production of either a toxic product such as an organic acid or a target-specific toxic agent such as an antibiotic.

Archaebacteria. A group of ancient bacteria which evolved very differently from other bacteria and are found today growing under very unusual environmental conditions (e.g., high salinity, high temperatures, methane production zones, and sulfur-rich conditions).

ATP. The abbreviation for adenosine triphosphate which forms a way of high energy storage in most living cells. Active bacteria would have a greater ATP concentration and shorter time lapses.

Attached bacteria. Bacteria which are able to attach directly to surfaces where reproduction and growth can occur while the cell(s) remain attached to that surface. Usually, these organisms grow within a biofilm.

Attachment. The process by which a microorganism can fix itself to a surface, usually by the excretion of extracellular polymeric substances (EPS).

Bacillus. A rod-shaped bacterium that can produce a heat-resistant spore.

Bacterial reactants. Bacteria which are able to set up either a physical or a chemical reaction within an environmental system to cause a reaction within that environment which will negatively affect the growth of competing organisms.

Bacteriophage. A virus that infects bacteria, sometimes referred to as a phage.

Bacteriostatic. Inhibits the growth and reproduction of bacteria.

BART-READ. A software program designed to allow the automatic testing of BART testers using either the single BART-type reader or the video reader that will monitor most BART tester types.

BART-SOFT. A software program that allows data on various BART testers to be entered and interpreted including zones of interrogation projection (ZIPs).

Bioamplifier. An organism which accelerates (i.e., amplifies) a natural physical and/or chemical reaction causing that reaction to occur at an accelerated rate.

Biocides. Specific compounds or groups of compounds which can, in either a gaseous, dry, suspended, or dissolved state, cause a toxic (lethal) effect on a broad spectrum of target organisms.

Biocolloid. A suspended organic particle in which living microorganisms may be surviving and/or growing. Size usually ranges between 8 and 100 μm. Density of biocolloids is similar to surrounding aqueous environment.

Bioconversion. The act of living organisms modifying substances to forms which are not subsequently (or normally) used for growth. These product forms may be recoverable. This is also known as biotransformation.

Biodegradation enhancement. A modification of the environmental conditions by techniques such as nutrient enhancement or inoculation of microorganisms to enhance the degradation of particular compounds of concern.

Biodetector. The name given to a device which allows the detection of specific or general presences relatable to various microbial activities.

Biodeterioration. Biologically mediated deterioration or destruction of materials such as paper products, woods, concretes, paints, and metals.

Biofilm. A continuous or discontinuous film of microbial growth occurring over a surface. Each of the individual cells is bound within a common matrix of extracellular polymeric materials (hydrogel). The biofilm may take the form of a thin "greasy" covering, a thick slime, or a complete plugging of the interstitial matrix within a given environment.

Biofilm dissolver. An organic or inorganic chemical or product of microbial activities which causes a biofilm to disintegrate and slough off into the associated liquid and/or other phases.

Biofilm-forming microorganisms. Group of microbes which are able to, either independently or in a consortium, form a continuous biofilm of either a temporary or permanent nature over a given solid surface at the interface with a water-based liquid matrix.

Biofouling. The phenomenon of deteriorations of any type resulting from biological activities (e.g., microbiologically influenced fouling); for example, the production of a thick biofilm leading to a tubercle with associated corrosion on an iron surface.

Bioincumbency. The density of viable microbial entities within a biofilm or a biocolloid.

Biomagnification. The accumulation of a substance within a living system beyond the basic requirement needs for the incumbent organisms. This is also sometimes referred to as bioaccumulation.

Biomass. The total mass of biological material (including any attached extracellular organic materials) that is present within a given system. In general, the biomass is estimated as a dry weight which normally forms

10%–20% of the wet weight of that given biomass. Carbon normally forms 50% of the total dry weight mass of the cells.

Biomass accumulation. An accumulation which occurs when there is an increase in biological growth (both in terms of cellular and extracellular product mass) as a result of growth. This biomass growth may occur in three forms: the planktonic (dispersed), sessile suspended (particulate), and sessile (attached) phases.

Biosensor. A device that allows the manipulation of a biological process to generate a signal (usually electrical) in the presence of particular substances (such as toxic agents).

Biozone. A localized formation of biological activity involving a specific group of organisms within a community structure. This may interface with other biozones.

Bleach. Common name for hypochlorite-based disinfectants widely used nowadays for the control of both planktonic and sessile bacterial populations. The most common forms used are sodium (liquid) and calcium (solid) hypochlorite and are generally considered to be particularly effective against the coliform bacteria.

Blended chemical heat treatment (BCHT). A patented process in which a severely biofouled water well can be regenerated by a triple-phased treatment involving the use of heat, disinfectants, and acidization to shock, disrupt, and disperse the biofouling.

Blended techniques. The utilization of more than one technique to control a given situation wherein the sum of the two control strategies when applied simultaneously is greater than that which would be achieved by using both techniques independently.

Campylobacter. Gram-negative, curved, rod-like bacteria which are considered to be one of the nosocomial bacteria which do occur on occasion in waters.

CB-4. A disruptive detergent that can effectively break up biomass at 0.5% concentration by degrading the EPS. At higher concentrations ($>0.5\%$), it becomes biocidal.

Chain of custody. The act of ensuring that a sample is taken, stored, shipped, analyzed, and disposed of in a manner that is fully documented.

Clogging. The total restriction in flow of groundwater through an aquifer and into a water well. This may be the result of the accumulation of materials such as chemical precipitates, silts, clays, and sands (see also plugging).

Coccus. A bacterial cell which may be spherical or close to spherical in shape.

Coliforms. Gram-negative, non-sporing bacteria which are facultative anaerobes and able to ferment lactose to acid and gas within 48 h at 35°C. Considered to be an excellent biomarker for fecal contamination due to the abundance with which these coliforms occur in fecal material. They are much less common in the natural environment.

Colony. The name given to a population of cells which grow as a cluster or assemblage on a solid surface sufficiently to become visible. Common surfaces used are agar gels or membrane filters.

Colony forming units (cfu). A method for the enumeration of the number of colonies formed on a given solid surface from a known volume or mass source. It is generally assumed that each colony unit formed represents one viable cell (i.e., each viable cell will form a separate and recordable colony).

Community structure. The form within which a consortium of microorganisms develops into a community structure in terms of the relative layering (stratified) positions of various species of bacteria one to another. It may also reference the various species within a community functioning interdependently.

Consortium. The name given to a group of organisms which work cooperatively to maintain a common eco-niche within which all can survive and flourish.

Continuous-flow slide culture. The cultivation of an attached biofilm on a surface under a continuously flowing liquid medium. These biofilm cultures can be used to determine the mechanisms of colonization of specific surfaces and subsequently interpret the dynamics of that growth.

Control strategies. Any strategy which has been designed and implemented in order to suppress a given biological problem without the expectation that the problem will be permanently resolved.

Corrosion. A process which relates to the deterioration of solid or porous materials and their surfaces by physical and/or chemical erosive mechanisms which can be catalyzed by the presence of biological amplifiers. For example, the production of hydrogen sulfide by sulfate-reducing bacteria can initiate electrolytic corrosion of steels often accompanied with tuberculation and the eventual disintegration of the structure afflicted (microbially induced corrosion). Acid-producing bacteria can cause an acidulytic form of corrosion.

Culture. The observable growth of specific microorganisms in a particular laboratory culture medium. Usually, the definition is restricted to a single strain or kind of organism.

Cyanobacteria. A bacterial group of photosynthesizing organisms in which oxygen is evolved. These used to be known as the blue-green algae.

Decomposer. The name given to an organism that breaks down complex organic materials into simpler components, including the releases of simple inorganic products.

Denitrification. The term used to refer to a reduction of nitrate to nitrite (partial) and to dinitrogen gas (complete) during anaerobic metabolic activities. This can cause loss of nitrogen (as a nutrient) to the ecosystem.

Detached bacteria. Any individual or group of bacteria which occur within the liquid phase having become detached from any solid surface.

These are commonly able to move either through motility or be carried along with the liquid flow possibly in biocolloids. These are sometimes referred to as planktonic bacteria.

Diagnostic. The act of determining the cause of a specific phenomenon. The methods may be chemical, physical, and/or biological.

Differential test. The test which is able, by some scientific means, to differentiate a sample into two or more possible groups.

Dinitrogen fixation. The microbial act of fixating gaseous dinitrogen (N_2) gas either aerobically or anaerobically into a form assimilable by the organisms.

Discharge. The act of water being pumped directly from the water well or seeping out from a spring or relief well.

Disinfection. The use of a physical and/or chemical process for the reduction in the total loading of microorganisms and the elimination of any (perceived nuisance) organisms thought to be associated as a water-borne concern.

DNA. The basic nucleic acid found in all living cells including microbes.

E. coli. The abbreviated form of *Escherichia coli*. This is a gram-negative, facultatively anaerobic enteric bacterium which forms one of the dominant bacteria in human feces and is the most frequently used marker organism for the indication of fecal pollution of food, surfaces, and waters; sometimes referred to as fecal coli (FC).

Electrolysis. A method for the generation of hydrogen and oxygen by the application of electricity. Several patents utilize this technique to control microbial events.

Encrustation. A thick irregular surface coating which usually has a high loading of inorganic compounds (particularly metallic salts which are being bioaccumulated). It is often fairly brittle in structure, forms sometimes as a senescent biofilm but still contain an active microbial component. Organic carbon content may decline to less than 0.5% of the total mass. Iron concentrations can reach as high as 98% in fully matured growths.

Endospore. A condensed form of an individual bacterial cell which is more durable, being resistant to heat and other harmful agents. It is a mechanism for survival and not reproduction.

Extracellular polymeric substances (EPS). Substances which are synthesized within a microbial cell and promptly excreted to form a tight capsule, loose sheath, or generally dispersed slime/gelatinous matrix within which the cell continues to metabolize and reproduce. The collective growth within a biofilm formed by EPS is sometimes referred to as the glycocalyx. Under some circumstances (e.g., biofilm sloughing or disintegration), the EPS can become dispersed into the aquatic phase as biocolloids.

Facilitative flow. A microbially induced flow which exceeds the rate observed under non-fouled (control) circumstances over a given medium.

Facultative. A qualifying adjective indicating that the organism is able to perform either in the presence or absence of the environmental factor being defined.

Fatty acid composition. A common term for the description of the microbial identification systems that use the relationships of the fatty acid methyl esters to each other within the composition of an unknown microbial cell culture to determine the most likely genus and species. These ratios can be used to accurately identify bacteria at the species level with a variation of less than 0.1% of any given fatty acid occurring within a species.

Fecal coliforms. See *E. coli*.

Fines. An engineering term that is applied to smaller particles generally in the micron or millimeter range of diameter that can perch within pores and fractures causing clogging. Fines include sand, clays, and silts, and are generally viewed as not having a biological component although frequently they do.

Fixed-film reactor. A system in which a chemical reaction is perpetrated by a biofilm actively growing in a fixed surface position within the reaction vessel that has been selected to perform that specific chemical function.

Food infection. A gastroenteric illness caused by the ingestion of microorganisms with food followed by growth which then initiates clinical symptoms of disease in the consumer.

Food intoxication. A poisoning of the consumer by microbial toxins produced in the food prior to consumption.

Food poisoning. A general term which references a gastroenteric disease involving the consumption of food contaminated with pathogens and/or their toxic products.

Gallionella. A gram-negative vibrioid to straight rod cell which excretes a ribbon-like twisted stalk out of one side of the cell. This stalk is rich in EPS polymers and the oxides and hydroxides of ferric, manganic, and other metallic elements. It is commonly believed to be a dominant component in iron-related bacteria consortia. This belief is reinforced by the distinct and easily recognizable iron-rich ribbon tail viewed in microscopic examination of the sample.

Generation time. The time required for a microbial population to double the number of cells through reproduction. This can range from as short as 20 min to as long as a number of days, weeks, or even years.

Genome. The complete set of genes (hereditary units) present in an organism.

Genus. A group of related species that possess common features.

Gradients. Defined changes in the concentrations of a substance which can occur over distance.

Groundwater microbiology. The study of any aspect of the phenomenon of microbial activities in and/or at the interfaces within groundwater systems at any depth within the crust. Major emphases at the present time

include the biodegradation of pollutant plumes (bioremediation), well plugging, and the assessment of the hygiene risks associable with water-borne infestations associable with groundwater sources.

Groundwater under the direct influence of surface water. Any groundwater that becomes impacted by surface waters moving down into the groundwater environment.

Halophile. The name given to an organism requiring high levels of salt (NaCl) for survival and growth (generally > 8% salt).

Dr. Hans Christian Gram. The inventor (1884) of the most successful differential stain for separating bacteria into two major groups (gram negative and gram positive). The Gram stain is universally applied to determine the reaction, arrangement, and morphology of bacteria. In that respect, it can be referred to as the Gram-staining technique.

Healthy carrier. An individual who harbors an infectious organism but does not display any diagnostic symptoms of infection.

Heterotrophic bacteria. Bacteria which are able to utilize organic materials as the principle sources of energy and carbon for survival, growth, and synthesis.

Heterotrophic plate count (HPC). A method for the determination of the numbers of heterotrophic bacteria in a water sample. When incubated at blood heat, significant counts can be considered to pose a potential health risk.

Hydrogel. A gel-like structure (such as slime) that has been generated by the EPS produced by the microorganisms. These hydrogels can also form encrustations and even forms of ice.

Hydrolysis. The act of breaking down of a chemical polymer into smaller structures (usually monomers) by the addition of water.

In situ treatment. Chemical and/or biological treatments being carried out at site in a particular environment (i.e., at the same position or site as the original process to be treated is functioning).

In vitro. Means occurring in culture under laboratory conditions.

In vivo. Means occurring within the body of a living organism or under natural conditions.

Incubation. Temperature at which an organism can be grown under suitable conditions. For microorganisms, the dominant factors are the culture medium, the temperature, and the composition of the gases surrounding the culture.

Induction. A process in which an enzyme is synthesized in response to the presence of an external substance (i.e., the inducer). Once synthesized, the enzyme will react with the inducer usually causing degradation. This process does take time which leads to a delay before the reaction can occur (induction period).

Infiltration intake. That point at which there is an infiltration (recharge) into a subsurface water reservoir through porous media.

Infiltrometer. An apparatus that measures the rate of infiltration of water into a porous medium using a descending or ascending head of water.

Invasiveness. The ability of a microorganism to enter an environment (or host), grow, reproduce, and spread throughout the domain.

Iron bacteria. The traditional name given to bacteria able to accumulate iron beyond the basic need for growth.

Iron-oxidizing bacteria. Bacteria capable of oxidizing iron to the ferric form, commonly an aerobic event.

Iron-precipitating bacteria. Bacteria which are able, during their normal life cycle, to cause the precipitation of iron salts inside or around the cell or within the aquatic environment.

Iron-reducing bacteria. Bacteria capable of reducing iron to the ferrous form, commonly an anaerobic event.

Iron-related bacteria (IRB). Any bacteriologically rich organic slime or encrustation which, when being generated, is rich in salts of oxidized iron due to bioaccumulation. These growths tend to be predominantly orange, red to brown. Color can turn to black under anaerobic conditions due to the sulfate-reducing bacterial activities. Also, it may be defined as any bacterium which can utilize iron within its life cycle in a defined manner whereby the oxides and hydroxides become either bound within the cell, within the EPS as a defined appendage, within the general capsule/sheath, or are precipitated in the environment around the growth. Iron-related bacteria forms an alternative title to the traditional use of the term "iron bacteria."

Koch's postulates. A set of rules for proving that a microorganism causes a particular event (such as a disease or degradation occurrence).

Lag phase. That period of time which extends from the inoculation of a population into suitable cultural conditions to the start of growth.

Legionella. Small gram-negative aerobic rods which are able to grow over a broad spectrum of temperatures and pH values. Sometimes occur in water distribution systems where the particulate organic carbon is above 0.6 mg/L. It also occurs in the intake/output lines from water heaters and is associated with water-borne infections causing Pontiac fever and Legionnaires disease.

Lipids. Alternative term for fats, which are water-insoluble molecules.

Low-nutrient environments (oligotrophic). Environments within which the nutrients (C, N, P, and S) are collectively very low, or where one element is sufficiently low for limiting growth. It should be noted that many "low-nutrient environments" may in fact support very heavy biofilm growths since the biofilm will act as a bioaccumulator of the nutrients even in aquatic systems where the dissolved nutrient levels are observed to be very low.

Luminometer. The name given to devices that allow the measurement of ATP as a primary indicator of microbial activity within the sample under test.

Macrofouling. Extremely intense forms of biofouling in terms of both activity levels and scale.

Magnetotactic bacteria. Bacteria which are able to position themselves within the magnetic fields. Specific magnetosome particles within the cell appear to be responsible for this orientation.

Marginal plugging. The phenomenon where well/groundwater have become biofouled and is subjected to sufficient biomass to reduce the rate of flow of water into, or out of, the water well. The level of flow reduction that may be expected with a marginal plugging would be less than 20% of the developed recorded flow.

Mechanical disruption. The use of abrasive forces such as brushing, rapid pressure fluctuations, ultrasonics, or rapid temperature changes from freezing through to steam generation in order to cause a mechanical disruption of the biomass.

Membrane filter (technique). The technique which uses a thin filter membrane to filter out bacteria (to produce a sterile filtrate) or to concentrate (on the surface to incubate and enumerate colonies that may grow) microorganisms that were present in the water sample.

Mesotroph. Any organism able to grow strictly within the temperature range of 15°C–45°C. Optimal growth is usually between 28 and 37°C.

Methylene blue. A crystalline dye used to determine microbial activities by changes in the color from blue to colorless as the medium becomes reductive. This dye is effectively a redox indicator with the threshold for bleaching (decolorization) appears to be when the oxygen concentration falls below 0.06 mg O_2/L. The rate at which this reduction occurs can be linked to the amount of microbial activity.

Microbial growth potential. The potential for any given environment to support the growth of microorganisms within the system based on the known environmental parameters for that system. For example, a high-nutrient environment with a mesotrophic temperature and pH 7.5 with saturated oxygen would be considered to have a high microbial growth potential.

Microcosm. A small portion of an environmental system that is separate and operates as a distinct biological unit. Usually involves very small volumes where conditions are optimized for the growth of a particular species or consortium of microorganisms.

Model water wells. Laboratory-scale replications (microcosms) of water wells wherein the flow rates, nutrient loadings, constructional methods, treatment systems, and microbial biofouling can be rapidly replicated and studied.

Models. Statistical predictive tools designed to utilize all of the valid information and generate an outcome. Commonly these models are used to project the hydraulic performance of engineered structures and commonly do not consider the potential for biological interferences with the performance of the model.

Most probable number (MPN). A statistical method for the prediction of a microbial population in a liquid by the presence/absence of growth in various dilutions of the sample.

Motility. The property of directional movement of a cell under its own power. Not to be confused with Brownian movement in which there is a random oscillation of the cell as a result of external influences.

Negative staining. A staining procedure in which the dye forms a dark background while the specimen (cell) remains unstained. It is convenient for examining the outline (shape) and arrangement of cells and also for observing EPS.

Nitrification. The aerobic conversion of ammonium via nitrite to nitrate. It is performed solely by the nitrifying bacteria which are usually classified using the prefix nitro- or nitroso- depending upon the function being performed.

Nodule. A dome-shaped growth commonly with a hardened crust that contains and protects a microbial slime that is growing within the dome.

Nosocomial bacteria. Bacteria which occur naturally in the environment but can, under some circumstances, cause infections in a suitably weakened host.

Nosocomial infection. The name given to an infection which is acquired and develops within a hospital or clinical care facility environment. Can also be called opportunistic.

Nutrient depletion strategy. The deliberate restriction of one or more nutrients from an environment in such a way as to attempt to control the growth of microorganisms within that system.

Obligate. A descriptive adjective referencing to all factors essential for the survival, growth, and reproduction of an organism (can also be called strict).

Occlusion. Scientific term meaning given to porous media or fractures that are plugged commonly with biomass.

Oligotroph. Name for any organism able to flourish in an environment having a low-nutrient regime.

Oxidation–reduction potential (ORP). A measure (in millivolts) of the electrically charged state of the groundwater. Generally, oxidative states (where oxygen is present) are positive and reductive states are negative (no oxygen or electron acceptors present). Oxidation–reduction potential is useful to determine the effectiveness of a well treatment.

Pandemic. A worldwide epidemic caused by a specific virulent pathogen.

Partial inhibition. The selective restriction of microbial activity by interference with the metabolic and/or reproductive functioning of the organism by manipulating the environment (e.g., application of low concentrations of disinfectant). It is intrinsically implied that the bulk of the microorganisms will eventually recover after a period of repair and adaptation.

Particulates. Suspended inorganic or organic defined materials formed into coherent masses with a diameter in excess of 0.5 μm. These particulates vary in diameters of up to 250 μm or more depending on flow characteristics. The origin may be from the sloughing of biofilms, direct

chemical reactions within the water, planktonic microbial growths, or any combinations of these.

Pasteurization. The process of heating a heat-sensitive substrate to just a sufficient heat to reduce the nuisance microbial population to an acceptable and manageable level. This process is usually performed within strict temperature and time constraints (e.g., 60°C for 30 min was Louis Pasteur's original standard).

Petri dish. A shallow glass or plastic vessel with a fitted lid. It allows microorganisms to be grown under aseptic (free from viable contaminants) conditions usually in agar culture media.

Planktonic. Used to define a microbial community growing as independent cells while suspended within the water and moving freely on the currents.

Plasmid. An extrachromosomal genetic element which is not essential to the growth of the organism but may contain information relating to unique additional functions.

Plugging. The phenomenon of an intense microbial biofilm growth (biomass) causing such a heavy slime generation that there is a significant ($>20\%$) loss in flow (hydraulic conductivity) through the interstitial spaces of a saturated porous medium. Used synonymously with the term clogging.

Plugging risk index (PRI). Defined as the specific utilization of the microbial growth potential within an environmental system to predict the rate at which biofouling and plugging are likely to occur. Factors to be taken into consideration include the basic physical and chemical parameters along with an evaluation of the likelihood of microbial infestations occurring.

Predicted active cells (p.a.c./mL). The predicted number of active bacterial cells per milliliter in a BART tester using the time lapse measured in seconds, hours, or days. Commonly, these predictions are calculated for tests incubated at 22 ± 1°C and standard interpretations are available on QuickPop version 3.1.

Preventative maintenance (PM). See also preventative servicing.

Preventative servicing (PS). Means that the well would be serviced in a routine manner to assure stabilization of the production characteristics of the well. This may be achieved by the management and application of treatments as may be deemed periodically necessary to prevent the significant recurrence of a significant microbially influenced problem. Preventative servicing should be a part of the design of the well and should take into account the likely factors that would need to be controlled and managed.

Pseudomonas aeruginosa. A gram-negative, strictly aerobic, rod-shaped bacteria which occur widely in the environment and can cause severe wound and postoperative infections.

Pseudomonas species. Strictly aerobic gram-negative rods which occur widely in groundwater systems, particularly where oxygen and/or nitrates are present in sizeable concentrations, associated frequently in IRB

communities, major contributor to organic biodegradation events. Several species are associated with nosocomial infections.

Psychrotrophs. Those organisms that are able to grow at temperatures below 15°C. They are differentiated into two groups. The first group is the obligate (or strict) psychrotrophs and will grow only over a range below 15°C. The other group is the facultative psychrotrophs which can grow usually much faster at temperatures above 15°C. The lower temperature at which activity can occur presently appears to be at around −15°C.

Q/s. Refers to the specific capacity as being a defined flow over a given distance in a time unit (seconds).

Recolonization. The act of the reestablishment of a microbial population (sessile and/or planktonic) within a given environment from which the population had been either eradicated or lost.

Redox front. The zone in, or around, the water well where the environment shifts from reductive to oxidative. It is at this front that most of the biomass will be generated.

Regeneration. The process of restoring an environment (such as a water well or groundwater system) towards its former (unfouled, pristine) state by the utilization of various treatments. Restoration and rehabilitation are alternative terms to regeneration that are also widely used in the industry.

Rehabilitation. See regeneration.

Reinfection. Involves the occurrence of a fresh infestation in a given installation by organisms either implanted by the maintenance crews or arriving at the water well after passage through the groundwater system or from surface recharges.

Repositioning. The strategy for avoiding a recognized infestation risk by relocating the plant equipment and water well installations away from the infestation zone.

Robbins's spool. A device which can be implanted into the walls of a given transmission or storage system for liquids or gases in order to monitor the rate of biofouling occurring by the patterns of colonization that occur. The device provides a mechanism for removing the colonizing biofilm from the system for evaluation and testing to diagnose the causant agents.

Roll over. The positioning of a small part of a transmission pipe for liquids (usually oils) at a lower level in order to build up turbulence and water activity to exaggerate any possible biological activities occurring within the pipeline as a whole (worst-case scenario).

Rusticle. The general name given to growths that form on iron-rich substrates (e.g., steel, iron ore) and forms a bioconcretion which has high iron contents ($>80\%$) and a diverse population of microorganisms. Commonly matures into various grades of pig iron.

Sample. Defined as that confined amount of the material collected for the purpose of being tested. It is usually considered that the sample would be representative of the site being investigated.

Sample storage. Relates to the method(s) that are employed to ensure that the sample remains a valid representative of the source site.

Scanning electron microscopy (SEM). The process of microscopy that uses reflected electron beams to form an image similar to that on the surface of any given material. By this means, it is possible to view biofilm formation, corrosion, and the effect that this is having on surfaces without destroying the surface growths.

Sequential mechanisms. Involve the utilization of a number of mechanisms in a specific sequence in order to ensure the maximum possible desired effect.

Sessile. Applied to cells or biomass that is growing attached to a surface.

Sheath. A hollow tube-like structure surrounding and protecting a chain of cells. Bacteria bearing this feature are sometimes referred to as the "sheathed bacteria."

Shock chlorination. The act of applying larger doses of a form of a chlorine (e.g., >5000 ppm of sodium hypochlorite) in order to maximize the disinfectant activity and reduce the presence and activity levels of the incumbent (fouling) organisms.

Slime. A jelly-like substance rich in EPS (hydrogel). Usually forms a coherent film covering walls, filling the interstitial spaces within the porous media such as that of aquifers, or coating well screen slots in a way to cause degeneration in the quality of the postdiluvial water through increased turbidity and decreases in the quantity of water flowing through a given system.

Slime bacteria. Those bacteria which reside and reproduce within the slime. The unique feature here is that the slime is capable of directed movement and the resident bacteria usually feed on other (commonly gram-negative) bacteria or cellulose.

Sloughing. The act of a biofilm (through stress events or as a result of flow, maturation, or pressure changes) disintegrating. This sloughing may cause layers of the biofilm to move to the suspended particulate phase detached from the rest of the biomass.

Specific capacity. See Q/s.

Spread plate. A technique which is applied in cases where the bacteria are to be enumerated. Generally, the sample is spread out over the surface of a suitable agar culture medium with a minimum of trauma. This is considered to heighten the probability of recovering and growing the incumbent organisms into distinct colonies.

Stabilization. A process which occurs with the development of an environment within which dynamics of the biological activity becomes stabilized causing no greater or lesser amount of problems over that given period of time.

Standard plate count (SPC). The term applied to recommended procedure for determining the number of bacteria (as cfu) in water.

Streak plate. A specific method for streaking a diluant of the sample over the surface of an agar plate to allow convenient enumeration of subsequent colonies that formed upon incubation.

Sulfate-reducing bacteria (SRB). Anaerobic bacteria able to reduce sulfate with the release of potentially corrosive hydrogen sulfide. They are sometimes referred to as sulfate-respiring bacteria. Sulfate-reducing bacteria are considered to be a major factor in the electrolytic corrosion of steels in the oil and gas industry.

Thermal death point (TDT). The lowest temperature required to destroy a microbial suspension in 10 min.

Thermal death time. The shortest period of time required to kill all of the organisms in a microbial population under specified conditions (e.g., temperature, cell age and number, volume, heat input, and carrier substrate).

Thermotrophs. Considered to be those bacteria which are able to grow only at temperatures in excess of 45°C. The upper limit for specialized thermotrophs has not yet been determined but exceeds 120°C and excessive pressures (e.g., >6000 psi).

Time lapse. The measured time that it took for particular samples to biologically generate a recognizable reaction. Time lapse is measured in seconds, hours, or days and relates inversely to microbial activity and population in the sample. Greater activity or larger populations would have shorter time lapses.

Total aerobic plate count. An evaluation of the number of aerobic (oxygen requiring or aerotolerant) bacteria within a given system by extinction dilution techniques commonly using spread plate techniques and temperature/incubation times optimized for the maximum population assessment.

Total coliforms. Gram-negative, facultatively anaerobic, oxidase negative, fermentative enteric bacteria which are either directly or indirectly associated with the pollution of waters by fecal material.

Total nitrogen (TN). An analysis for all forms of nitrogen within water. Fractions include nitrate–nitrogen, ammonium–nitrogen, and Kjeldahl nitrogen. While the former states are considered soluble, the latter state relates to nitrogen bound into various forms in biological material.

Total organic carbon (TOC). The total organic carbon recoverable in a sample. Fractions may include soluble utilizable organic carbon (SUC), soluble non-utilizable organic carbon (SNUC), total non-utilizable organic carbon (NUC), particulate utilizable organic carbon (PUC), and the particulate non-utilizable organic carbon (PNUC). The ratios of SUC:SNUC and PUC:NUC will give an indication of the degradability of organic pollutants within the system.

Total phosphorus (TP). The total amount of phosphorus recovered from an aquatic system including the soluble inorganic phosphorus (SIP), particulate inorganic phosphorus (PIP), particulate organic phosphorus (POP), and the soluble organic phosphorus (SOP). The interactive ratios

between the SIP:PIP:POP and SOP can be used to determine the amount of biological activity occurring.

Treatment fringe effect. The phenomena generated on the fringes of a given physical/chemical/biological treatment zone. At the lower treatment dosages occurring at the fringe, marginal effects may be seen wherein the microbial growth may become partially suppressed, stimulated, or be unaffected by these particular factors.

Tubercles. Raised encrustations on a surface within which a biofilm may be generated. These growths are frequently associated with electrolytic corrosion.

Tyndallization. The suppression of a microbial growth and the repeated application (normally three times) of heat (pasteurization) in which sufficient time is allowed between treatments for the surviving organisms to grow to a point that these survivors now grow to become more vulnerable to heat (i.e., the next treatment).

Ultramicrobacteria (UMB). The name given to microbial cells that have undergone severe environmental stress and have reacted by entering a phase of suspended animation (dormancy, sleep). Where this happens, much of the cell contents and water are either expressed from the cell or utilized so that the diameter of the cell shrinks to between 0.1 and 0.5 μm. As a final action, stressing this cycle, the cells will reproduce. These cells remain fully viable and will grow again rapidly when the environmental conditions are appropriate. Ultramicrobacteria are unable to attach to surfaces. There is evidence that UMB can survive in a dormant state for millions of years.

V-BART-READ. A tester video camera logging and interpretation system that allows the simultaneous monitoring of eight BART testers. This reader uses temperature controlled at 22 ± 1°C and the camera connects via a universal serial bus (USB) connector to a computer for storage, and the interpretation is by using V-BART-READ software.

Viable counts. A procedure which involves the enumeration of microorganisms specifically restricted to those cells known and recognized to be viable and shown to be active.

Vyredox®. The trademark for a patented system for reducing the plugging of water wells and/or improving the water quality by oxidizing out the iron and manganese in situ away from the well screen. The method effectively shifts the redox fringe back into the aquifer by creating a larger oxidative zone.

Water-borne infections. Generalized name applied to those infections created by pathogens which have reached the host via water.

Well pasteurization. Utilization of high temperatures (in excess of 60°C for 30 min) in order to destroy the biomass formed by microorganisms at the water well/groundwater interface.

Wolfe's medium. A specialized selective medium for the growth of *Gallionella* used in the diagnosis of iron bacterial infestations within wells.

Winogradski-Regina (WR) medium. A modified form of Winogradski medium developed at the University of Regina (hence WR), which appears to be able to enumerate the bulk of the IRB.

ZIP. Zones of interrogation projection designed to be able to examine the various microbial communities growing in, and around, the well. BART-SOFT version 5.0 includes a program which allows ZIPs to be generated for a well by sampling and testing with BARTs during a pump test.

S Well Questionnaire (First Enquiry)

It is important to obtain background information on a well before recommending a proposed management strategy to the client. At the end of this appendix is the simple questionnaire that is used by Neill and Gunter, Design and Consulting Engineers, as the first stage in developing a suitable approach for the client. This questionnaire is very general in nature but covers the many basic issues associated with well construction, hydraulics, geology, and chemistry. From the answers to these questions, it is possible to diagnose well efficiency issues and then develop a strategy which might include more testing prior to well regeneration or the onset of a program for the preventative maintenance servicing of the well in question. This questionnaire therefore allows the first-level evaluation of the well in question. Note that only two questions relate to the potential microbiological challenges for that well (Well water microbiology? and Any indication of iron bacteria?). This is a deliberate strategy in the questionnaire since within the profession there is not yet a major understanding of the manner in which microorganisms can impact on the performance of biofouling water wells.

Only a very small percentage of water wells would normally have only limited information on the well water microbiology. In general, the information might be limited to the presence or absence testing for total and/or fecal coliform bacteria (associated with potential health and hygiene risks) and maybe some general cultural data presented in colony forming units per milliliter and performed using agar spreadplate methodologies. This question would therefore lead to the need to perform more selective tests for particular groups of bacteria that could be directly linked to plugging, corrosion, and water quality issues. Blanket testing to determine the level of bacteriological activity could be done using the various forms of the adenosine triphosphate (ATP) test, which can be performed at the well head and give an indication of the ATP loading in the well water. This loading is recorded as relative light units (RLU) and can range from as low as a few hundred to as high as 100,000 or more. Manufacturers of the luminometers supply various formulations for converting the RLU to ATP. This form of ATP testing determines how much microbiological activity is occurring in that well water sample but does not differentiate the causes of that activity. Causes of the activity can be determined using the biological activity reaction test (BART) testers and systems in which the activity is determined in seconds, hours, or days for specific groups of bacteria. Additionally, sequential pump sampling can be used along with the BART testing to

determine where the different bacterial communities (consortia) are active in, and around, the well. This is done by using the zones of interrogation projection (ZIP) software program. This, in concert with the ATP, can now give the client and the consultant a realistic idea of not only where the active nuisance bacteria are located, but also what the biofouling risks are to that well and where the biomass is located. In designing water well regeneration treatments or an ongoing preventative servicing scenario for wells, it is critical to understand which microbes are the most active and where these are located in relation to the borehole and surrounding formations. By developing a strategy of ATP testing along with BART evaluations, it is possible to develop the ZIP that indicates where the biomass is distributed so that suitable treatment strategies can be developed. It must be remembered that it is not possible to "sterilize" the well in any practical manner because of the ease with which reinfestations can occur from the groundwater in the surrounding formations. This means there will be ongoing biomass growth and replugging if well servicing is ignored. Effective design and engineering can assure the client that as a minimum the biofouling can be held in check and the specific capacity will be stabilized over a longer period of time.

The second question "Any indication of iron bacteria?" provides a different approach to the client. This determines whether iron has been perceived to be a problem through the formation of iron-rich encrustations (popularly termed ochers), production of slimes, nodules, or tubercles rich in iron, or simple iron in the water (yellow, orange, browns, or even dark greens). This means, for the client, that the well should be subjected to video camera logging to determine where these iron-rich growths are occurring if they are visible at all on the screen or inside the well. The client needs to be aware of the importance of the video camera logging of the well and also that if it shows no signs of heavy microbial growth it does not mean that the well is free from such biomass. It could be that the biomass is all away from view through the slots, fractures, and perforations, and so now it is even more important to undertake BART testing for bacteria including the iron-related bacteria, sulfate-reducing bacteria, heterotrophic aerobic bacteria, slime-forming bacteria, and denitrifying bacteria. Using the ZIP approach, it would be possible to determine the locations of these various bacteria in the well beyond the vision of the camera. The accumulation of iron in ochers, slimes, encrustations, nodules, and tubercles all are strong indicators of the presence of active bacteria that could then also be associated with the plugging, corrosion, and water quality risks for the well and adjacent geological formations. This questionnaire therefore provides a first line of inquiry that can then be used to build up an effective management strategy for the well in question.

WELL REGENERATION INFORMATION

Well Construction

Well depth:_____

Well diameter:_____

Casing depth:_____

Well screen length:_____

Well screen location:_____

Any other modifications:_____

Well Hydraulics

Present use of well, hours per day:_____

Present use of well, days per year:_____

Present well discharge/production or yield:_____

Historical well discharge/production or yield (data or graph):_____

Present pumping water level:_____

Historical pumping water level (data or graph):_____

Present static water level:_____

Historical static water level (data or graph):_____

Distance to closest well:_____

Past well maintenance:_____

Geology

Well log available? (if yes, attach):_____

General lithology of area:_____

Is this a sand and gravel aquifer?:_____

Is this a bedrock aquifer?:_____

How thick is the aquifer?:_____

Nearby water bodies and proximity:_____

Well water chemistry

Is general chemistry of water available? (if yes, attach):_____

General water chemistry issues in this area:_____

Field parameters such as temperature, conductivity, pH, dissolved oxygen:_____

Well water microbiology:_____

Any indication of iron bacteria?:_____

Other comments:

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